INTEGRATIVE NEUROBIOLOGY AND BEHAVIOR OF MOLLUSCS

Organized by

ROGER T. HANLON
MARINE BIOMEDICAL INSTITUTE
UNIVERSITY OF TEXAS MEDICAL BRANCH
and
ALAN M. KUZIRIAN
MARINE BIOLOGICAL LABORATORY

AMERICAN MALACOLOGICAL UNION
WOODS HOLE, MASSACHUSETTS

4 - 5 JUNE 1990

Sexual conflict and the mating systems of simultaneously hermaphroditic gastropods

Janet L. Leonard*

Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019, U. S. A.

Abstract. Here I review the predictions, for hermaphroditic gastropods, of recent developments in mating systems and sexual conflict theory. Sexual conflict theory predicts that hermaphrodites should have a species-specific preferred sexual role. The Hermaphrodite's Dilemma model explores the consequences of this and predicts that all hermaphrodite mating systems should be based on reciprocity with cheating in a preferred role. Traditional models based on Bateman's principle predict that the male role will be preferred. Experimental and observational evidence from *Navanax inermis* (Cooper) indicate that the female role is preferred, contrary to predictions from Bateman's principle, and that the mating system is based on sperm-trading which serves to enforce reciprocation, preventing individuals from specializing in the female sexual role. Comparison of *Navanax* to other hermaphrodites suggests that the preferred sexual role is that which offers control of fertilization. This, the gamete-trading model, predicts that all hermaphroditic gastropods with sperm storage and a gametolytic gland should demonstrate a preference for the female role and a mating system based on sperm trading. This model and the Hermaphrodite's Dilemma model of strategies in a situation of sexual conflict make specific predictions about the behavior of hermaphroditic gastropods. The available literature on opisthobranchs and pulmonates suggests several interesting tests of these models but the available data are insufficient to support or refute the predictions. The mating systems of euthyneuran gastropods require investigation from the standpoint of modern mating systems theory.

Recent progress in understanding the evolution of species-typical reproductive behaviors stems largely from the analysis of mating systems in terms of a conflict of interests between the sexes (Orians, 1969; Trivers, 1972; Emlen and Oring, 1977; Parker, 1979; Hammerstein and Parker, 1987). Because such sexual conflict is assumed to be a product of the differential selective pressures associated with reproduction through sperm versus reproduction through eggs (Parker, 1979), it should exist in all anisogamous organisms (Bateman, 1948). Although work in this field (despite Ghiselin, 1974; Williams, 1975) has concentrated on a small number of taxa (largely vertebrates and insects), almost all of which have separate sexes, there has been increasing interest in the role of sexual conflict and/or sexual selection in the mating systems of a wider array of organisms, such as plants, including hermaphroditic forms (Willson and Burley, 1983; Bronstein, 1988; Galen and Rotenberry, 1988; Nakamura and Stanton, 1989) and some animals with sequential hermaphroditism (primarily fish, see Charnov, 1982, 1986; Shapiro and Boulon, 1982; Warner, 1982; Warner and Lejeune, 1985) and more recently simultaneous hermaphroditism (e.g. serranid fishes; Fischer, 1980, 1984; Fischer and Petersen, 1987; and the polychaete, *Ophyotrocha*; Berglund, 1985; Sella, 1985, 1988).

Extension of the analysis of sexual conflict to a diverse array of taxa and modes of sexuality is important for two reasons: 1) it could provide new and useful insights into the biology and evolutionary ecology of taxa of interest; 2) by examining the mating systems of a diverse array of organisms in terms of sexual conflict and/or sexual selection, we should be able to identify useful systems for testing some of the assumptions and predictions of modern mating systems theory.

The gastropods are particularly interesting in this respect because they offer: 1) a diverse array of sexual systems and reproductive strategies; 2) a variety of very complex reproductive behaviors; 3) complex genitalia, the anatomy of which is important taxonomically in many groups. Where genital anatomy is varied sufficiently to be a useful taxonomic character at the levels of genus, subgenus and species, as in some groups of gastropods (Mead, 1943; Rudman, 1974; Gilbertson, 1989; Patterson, 1989), sexual selection is likely to have been important (Eberhard, 1985). Similarly, where there are elaborate forms of courtship and copulatory behavior, and particularly where there is significant diversity within a taxonomic group, one predicts that sexual conflict and/or sexual selection has been important as a selective force.

In this paper, I 1) present predictions as to the types of sexual behavior and mating systems expected for hermaphroditic gastropods if sexual conflict is important and 2) discuss a few well-known sexual phenomena in the two predominantly hermaphroditic subclasses of gastropods (Opisthobranchia and Pulmonata) in light of predictions of

---

*Address correspondence to: Mark O. Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365, U. S. A.

recent models based on the assumption of sexual conflict. These two subclasses, collectively (loosely) termed the euthyneuran gastropods, are predominantly outcrossing simultaneous hermaphrodites. Hermaphroditic species offer exciting opportunities to test: 1) the hypothesis that sexual conflict exists (Leonard, 1990), because as Parker (1979) has pointed out, sexual conflict has been widely assumed but there is little evidence that it exists; 2) alternate hypotheses as to the source and nature of sexual conflict (Leonard and Lukowiak, 1991).

SEXUAL CONFLICT

SEXUAL CONFLICT AND HERMAPHRODITE MATING SYSTEMS

Sexual conflict is a conflict of interests between the two parties to a mating encounter such that one individual has more to gain (less to lose) by mating than the other does. The idea of sexual conflict is based on the common observation that among many species of animals, males are ''eager'' to mate with virtually any available female, to the extent of risking death and/or serious injury in fighting other males for access to females, whereas females are ''coy''. This phenomenon, which Darwin (1874) considered paradoxical, is usually explained by Bateman's (1948) principle, i.e. that males are more eager to fertilize eggs than females are to get their eggs fertilized because the fitness of females is limited by the resources available for egg production, whereas the fitness of males is only limited by the availability of females.

For a simultaneous hermaphrodite, sexual conflict arises if and when there is more to be gained from mating in one sexual role than in the other. In the population as a whole, reproductive success through eggs must exactly equal reproductive success through sperm (R. A. Fisher, 1958). However, the distribution of reproductive success across the population could well differ for sperm and eggs (i.e. the variances differ, see Charnov, 1979). When this is the case, there is a potential asymmetry in the pay-offs of the two sexual roles to an individual, particularly in a single encounter, and an individual's overall fitness (its reproductive success relative to the rest of the population) will depend in part on how it divides its reproductive effort (or reproductive opportunities) between the two sexual roles. That is, an individual which specializes (differs from the population average of 50:50 reproduction through sperm versus eggs) in one of the roles (that which is less costly in terms of energy expenditure, mating time, risk, etc., see below) could be able to achieve greater than average fitness. This means of course that other individuals in the population will find themselves specializing inadvertently in the more costly role, which should result in below average fitness.

Charnov (1979:2482) discussed the implications of Bateman's principle for pair-mating hermaphroditic animals, including gastropods, and recognized that, in simultaneous hermaphrodites; ''There must often be a conflict of interest between mating partners — as a recipient each should be inclined to accept sperm (not necessarily for fertilization of its own eggs) in order to give its sperm away. As a donor, one should be selected to induce one's partner to use the new sperm in fertilization''. He went on to suggest that both the complicated reproductive anatomy and the elaborate precopulatory behaviors of animals such as gastropods ''are explicable when one realizes that the interests of the partners are often in conflict''. In hermaphrodites this sexual conflict is direct, in that each individual is in direct competition with all other individuals, including its mate, for fitness. On the other hand in dioecious species sexual conflict (with the exception of conflict over parental care) is an epiphenomenon of intrasexual competition (Hammerstein and Parker, 1987).

Thus, in simultaneous hermaphrodites sexual conflict should lead to the evolution of a preference for mating in a particular sexual role, a preference that will be shared by all individuals of the species. If all individuals prefer the same role, the interests of two individuals meeting for a mating encounter will be in direct conflict. In hermaphrodites, each mating encounter could be expected to involve competition between members of the pair for the preferred role. Mating systems in hermaphrodites should reflect or represent a resolution of, this competiton for the preferred role (Leonard, 1990, unpub. data; see also below).

THE ORIGIN OF SEXUAL CONFLICT

If sexual conflict exists, the preferred sexual role will be consistent within a species, since the term implies an inherent advantage to one sexual role, but the favored role could vary between species, depending on the source of sexual conflict. Charnov based his arguments (Charnov, 1979, 1982) on the explicit assumption of Bateman's principle, i.e. that male fecundity is limited by access to eggs while female fecundity is not limited by sperm availability. While Bateman's principle is the most widely accepted explanation of sexual conflict, there are two general types of alternative explanation: mating time or rate (Baylis, 1981; Sutherland, 1986); and control of fertilization or risk (Alexander and Borgia, 1979; Leonard and Lukowiak, 1984, 1985, 1991). The first of these, the rate hypothesis, argues that a discrepancy between males and females in the minimum interval between matings, could be a source of sexual conflict. That is, if after a mating encounter, one sex (say the female) must spend a prolonged period in yolking up eggs, or some form of parental care (gestation, brooding, etc.) before being able to increase her fitness by a second mating encounter, whereas the male can inseminate many females in that same time period then one could expect the female to be ''coy'' and the male, ''eager'', even in the absence of differential energy expenditure. For a discussion of how mating rate can favor the evolution of male parental care see Baylis (1981).

The second type of hypothesis is based on the idea that the important dichotomy between reproducing through eggs and reproducing through sperm may not be differential costs in a fixed currency (such as energy or time) but rather a differential probability that the investment that is made will pay off in the form of zygotes. If gamete production involves use of an exhaustible resource, then in economic terminology, optimal depletion of that resource (i.e. depletion which maximizes profit) must be based not only on accounting costs (the energy, time, etc. required for a particular type of reproductive act) but also the opportunity cost (that is the cost of a reproductive act now must include the value of that act if it were made at some future date, or the pay-off that could have been achieved from the other role, etc.) (Nicholson, 1978; A. C. Fisher, 1981). Alexander and Borgia (1979) suggested that an important difference between the sexes could be the extent to which control is exerted over the fate of the gametes that they produce. Alexander and Borgia (1979) argued that females, in general, produce fewer gametes not because they are limited by energy availability but rather because they invest more in "following-up" on that gamete, insuring that it will be fertilized. That is, one can think of females as adopting a "risk-averse" reproductive strategy whereas males have a "risk-prone" reproductive strategy. This type of model has some interesting implications for hermaphrodites.

The unique feature of hermaphrodite sexual strategies is obviously the opportunity to choose between reproduction through eggs or sperm. If, as is required for sexual conflict, the variances of these two types of strategy differ, which should be preferred, the high variance strategy or the low variance strategy? Extension of Bateman's principle to hermaphrodites (Bateman, 1948; Charnov, 1979) suggests that the male role (the high variance strategy) will be preferred (see above). However, Gillespie (1977) has demonstrated that where two genotypes yield equal average offspring number, but have unequal variances, the one with the lower variance will offer a fitness advantage. This suggests that given an opportunity to choose between a high and a low variance reproductive strategy, selection would favor the individual that opted for the low variance strategy (Leonard and Lukowiak, 1991). In Gillespie's (1977: 1012) words, "the addition of a stochastic element to the offspring number of a genotype will effectively lower the fitness of that genotype as measured by its mean frequency in the next generation". A practical problem in using Gillespie's principle to predict the favored sexual role for a hermaphroditic species is that we have little empirical evidence as to the relative variances of offspring production through male versus female function. In general, however, male reproductive success has been assumed to have the greater variance. Under this assumption, predictions from Gillespie's principle would be very different than those from Bateman's principle.

The gamete-trading model (Leonard and Lukowiak, 1984, 1985), developed from a comparison of the mating systems of the aglajid opisthobranch, *Navanax inermis* (Cooper) (Leonard and Lukowiak, 1984, 1985) (for discussion of the validity of the genus see Rudman, 1974; Gosliner, 1980), and a serranid fish (Fischer, 1980), is based on the premise that the preferred role for a simultaneous hermaphrodite will be the one that controls fertilization, i.e. is the last to make an irrevocable commitment of gametes. The sexual role that controls fertilization affords the greatest certainty that the investment made will result in zygotes. That is, the preferred role will have the greatest certainty of parenthood. Considered from the standpoint of selection if two reproductive options will have the same mean pay-off but one is less risky than the other, an individual with limited resources to invest will do better to play it safe (Gillespie, 1977; Philippi and Seger, 1989).

Such competing hypotheses as to the source of sexual conflict can be tested in hermaphrodites by finding species for which two of the hypotheses make opposite predictions as to the sexual role that should be favored and then determining which role is in fact preferred. For example, experimental studies designed to test the conflicting hypotheses that in *Navanax inermis*, the male role is preferred as would be expected from Bateman's principle and the egg-trading model (Fischer, 1980; see discussion in Leonard and Lukowiak, 1991), versus the conflicting hypothesis that the female role is preferred (based on the gamete-trading model), indicate a preference for the female role (Leonard and Lukowiak, 1991). In this way, the debate over the factors responsible for sexual conflict can be moved from a theoretical level to an experimental one.

DOES SEXUAL CONFLICT SHAPE MATING SYSTEMS?

The question of the importance of sexual conflict in shaping mating systems could be best answered by comparative studies. That is, we need to decide whether the mating systems observed in simultaneous hermaphrodites conform to what would be expected if sexual conflict were important. A model in the form of a game of strategy, termed Hermaphrodite's Dilemma, has been developed to analyze the situation that arises given a conflict of interest between two simultaneous hermaphrodites in a mating encounter (Leonard, 1990). This model can provide qualitative predictions as to the type of behavior and mating system to be expected under a variety of conditions.

HERMAPHRODITE'S DILEMMA

Briefly, Hermaphrodite's Dilemma is a two-person, non-zero-sum, conditional game of strategy; the available decisions are: 1) to offer to assume both roles in a mating encounter (the cooperate decision), or 2) to mate only in the preferred sexual role and then desert without allowing the partner to mate in the preferred sexual role (the defect decision). Assuming that both players are, being simultaneous hermaphrodites, prepared to mate in both roles at all times, and that there is an advantage to mating in one role, an individual ought to be willing to assume that role in any and all encounters. Therefore, its decision in a mating encounter is not whether or not to mate in the preferred role, but whether or not it should also mate in the non-preferred role. The model predicts that the best strategy over a wide variety of conditions will be one that combines cooperating (reciprocation) most of the time, with a certain low level of defection (= cheating), which could or could not be contingent on the partner's prior behavior, depending on circumstances [i.e. w, the probability of encountering a partner again, (Axelrod and Hamilton, 1981), and whether the pay-off matrix corresponds to Prisoner's Dilemma or Chicken]. That is, given sexual conflict, the mating system of simultaneous hermaphrodites should be based on reciprocation, with cheating in a species-typical preferred sexual role occurring at a relatively low frequency, and the existence of mechanisms to reduce vulnerability to cheaters (Leonard, 1990).

Furthermore, the model suggests that mechanisms should exist to prevent cheating and/or "punish" cheaters. Although the arguments developed here should apply to all hermaphroditic gastropods, the discussion will focus on the pulmonate and opisthobranch (lumped here as "euthyneuran") gastropods because they offer a wide array of forms of (often bizarre) reproductive behavior [communal parental care (Rose and Hoegh-Guldberg, 1982), hypodermic copulation (Rivest, 1984), chain copulation (*Aplysia* and other species), and elaborate and bizarre forms of courtship (*Helix, Limax* and some other stylommatophorans)] that have been little studied. Even the familiar cases (i.e. *Helix, Limax, Aplysia*) have not been studied in light of modern mating systems theory. The analysis presented here suggests new interpretations of familiar phenomena in gastropod biology and proposes tests of important assumptions and predictions of mating system theory using comparative and experimental studies of these gastropods.

The Hermaphrodite's Dilemma model predicts that where unilateral copulation is the rule, 1) mechanisms for enforcing reciprocation exist [such mechanisms could include explicit alternation of sexual roles, such as that described for *Navanax* (Leonard and Lukowiak, 1984, 1985)], 2) effective reciprocity is achieved by random mating within a small mating group [there is some evidence to suggest that *Aplysia californica* Cooper form small, relatively stable mating clusters (Kupfermann and Carew, 1974)], or 3) willingness to copulate in the less preferred role is maintained by the conditions of the Chicken matrix (Riechert and Hammerstein, 1983). For example, *Lymnaea stagnalis* (L.) are always ready to copulate as females, the preferred role according to the gamete-trading model, but become willing to copulate as males after periods of isolation (v. Duivenboden and ter Maat, 1985). Alternatively, if the mating system were not based on reciprocation, one would expect to see aggressive attempts to force copulation by individuals acting in the favored sexual role, with the victim attempting to avoid copulation and/or to retaliate by assuming the favored role, as could be the case in some leeches (Leonard, unpub. data).

The problem faced by a pair of simultaneous hermaphrodites in a mating encounter differs from that described in the classical Prisoner's Dilemma (Luce and Raiffa, 1957; Davis, 1983) or in the formal Hermaphrodite's Dilemma model (Leonard, 1990) in that a player has information about the decision made by its partner, and in general, one player will have to make the first move. One possible tactic in this situation would be the "Quick-Draw" approach; making the first move and assuming the preferred role, leaving the partner to either assume the non-preferred role or pass up the chance to mate. If the partner agreed to assume the non-preferred role, it would be possible for the initiator to either "cheat" (by leaving without reciprocating) or to reciprocate, by offering to mate in the non-preferred role also. This type of tactic could result in the evolution of either 1) "Hit-and-Run" mating encounters, such as those of some leeches (see above), or 2) insistence by the party of the second part on strong assurance that the initiator will reciprocate, before the party of the second part assumes the non-preferred sexual role. The simultaneous reciprocal copulation found in many nudibranch and stylommatophoran gastropods and clitellate annelids (earthworms and some leeches) could have evolved in this way.

In species in which mating is not (or cannot be) simultaneously reciprocal, an individual pursuing a strategy such as Tit-for-Tat should advertise its willingness to reciprocate, in order to attract potential partners or to avoid rejection by a partner that it has located. Assuming the non-preferred sexual role in the first mating would be a way of accomplishing this. For such a tactic to be successful, there would have to be some protection against "cheating" by the partner. Such tactics appear to be employed as part of a Tit-for-Tat strategy in the mating systems of certain serranid fishes (Fischer, 1980, 1984) and at least one opisthobranch gastropod, *Navanax inermis* (Leonard and Lukowiak, 1984, 1984, 1991).

EUTHYNEURAN GASTROPODS

GENERAL REPRODUCTIVE BIOLOGY
(for review see Tompa *et al.*, 1984)

All but a very few species of the euthyneuran (Subclasses Opisthobranchia and Pulmonata) gastropods are simultaneously hermaphroditic. Among euthyneuran groups, gonochorism is common only in the opisthobranch order Acochlidioidea (Hadfield and Switzer-Dunlap, 1984; but see Wawra, 1988). While many authors refer to protandry in these taxa, this usually means only that histological investigations show mature sperm present before eggs have matured. For example, in *Limacina*, an opisthobranch considered to be protandrous, copulation appears to be simultaneously reciprocal between mature males (Lalli and Wells, 1978; other examples in Hadfield and Switzer-Dunlap, 1984). This usage has been common in the molluscan literature since at least the end of the last century (e.g. Pelseneer, 1895). Storage of allosperm (and often autosperm) is common and individuals could receive sperm which they will later use to fertilize eggs, long before eggs have been formed in the ovotestis. True sequential hermaphroditism, in which an individual is purely a sperm donor at one stage of its life and only a sperm recipient at another, is rare, if it in fact exists, in euthyneuran snails (Geraerts and Joose, 1984; Hadfield and Switzer-Dunlap, 1984; Tompa, 1984), although it is not uncommon in the prosobranch gastropods. One must be cautious, therefore, in interpreting references to functional protandry (see discussion in Ghiselin, 1965).

Reproduction through true parthenogenesis (probably automitic) has been reported for one stylommatophoran slug (Hoffmann, 1983; see also discussion in Tompa, 1984). Although the capacity for self-fertilization is not uncommon in pulmonates, and apparently exists in a few opisthobranchs (Hadfield and Switzer-Dunlap, 1984), cross-fertilization is the rule and the vast majority of euthyneuran gastropods act as simultaneous hermaphrodites throughout their reproductive lives.

The anatomy of the reproductive system of euthyneuran gastropods can be summarized as very complex, highly diverse, and taxonomically valuable at the species level. As Eberhard (1985) pointed out, these characteristics are indicative of rapid evolution, probably as a result of sexual selection. This, in turn, suggests that these gastropods should be characterized by diverse, complex and fascinating sexual behavior and mating systems. Unfortunately, our understanding of the reproductive biology of these gastropods is very scanty. For most species only the gross anatomy of the genitalia has been described; less is known at the histological level, and there have been relatively few studies that have looked directly at the physiology of various parts of the reproductive tracts. In many cases it is difficult to imagine how the genitalia would look when everted and how the various parts would fit together during copulation (see Reeder, 1986). Also, most of the available information on sexual behavior consists of casual or anecdotal observations.

In terms of sexual behavior, euthyneuran gastropods can be divided into three groups; those in which copulation is normally simultaneously reciprocal, those in which it is unilateral, and those in which copulation is unilateral and chains of copulating individuals occur commonly. As a broad genralization, simultaneous reciprocal copulation occurs in taxonomic groups in which the penis and common genital aperture (= vaginal pore) are close together on the body, while unilateral copulation is typical of taxonomic groups in which these structures are widely separated. Speaking again very generally, one can say that simultaneous reciprocal copulation is characteristic of the stylommatophoran pulmonates (exceptions include *Stenotrema*, Webb, 1948), whereas the basommatophorans have unilateral, and sometimes chain, copulation (Geraerts and Joose, 1984; Tompa, 1984). Among the opisthobranchs, simultaneous reciprocal copulation (and/or sperm transfer) is found in most of the nudibranchs, notaspideans, saccoglossans and pteropods, whereas unilateral and/or chain copulation (or sperm transfer) is more common in cephalaspideans, anaspideans, pyramidelloideans and acochlidioideans (Hadfield and Switzer-Dunlap, 1984). True copulation is the general mode of sperm transfer in euthyneuran gastropods, but hypodermic insemination and aphallic spermatophore transfer have evolved several times among opisthobranchs (Hadfield and Switzer-Dunlap, 1984). Some land slugs deposit sperm on the tip of the partner's penis (Gerhardt, 1933; Tompa, 1984). Aphallic sperm transfer apparently occurs in some basommatophorans (Geraerts and Joose, 1984). In the nudibranch *Aeolidia* sperm are deposited on the outer surface of the female gonopore (Longley and Longley, 1984).

OPPORTUNITY FOR SPERM COMPETITION

SPERM STORAGE

Although the functions of the myriad organs of gastropod reproductive systems are poorly understood, one can say that both opisthobranchs and pulmonates typically have organs for storage of allosperm and there is some evidence that allosperm could require a period of residence in the sperm storage organ before becoming competent to fertilize eggs (Hadfield and Switzer-Dunlap, 1984). The period of residence in the sperm storage organ is uncertain for most species. Hadfield and Switzer-Dunlap (1984) reported that while some opisthobranchs show a close association between copulation and egg laying, with egg laying following copulations at a fairly predictable interval, this is not always the case. Individuals could copulate many times without spawning. One *Navanax inermis*, under laboratory conditions, copulated 24 times in the female role before laying an egg mass (Leonard and Lukowiak, 1985). The record for the number of egg masses laid between copulations is apparently still held by an *Aplysia californica* taken to Woods Hole, Massachusetts, and held in isolation by MacGinitie (1934). That individual laid eggs for more than four months and the first 15 egg masses were fertile, demonstrating that *Aplysia* can store sperm for at least two and one-half months and that the sperm storage organ can hold enough sperm to fertilize at least 15 egg masses.

In *Navanax*, one isolated individual laid nine egg masses over the course of a month. The first five egg masses developed normally; the last four were inviable. After being allowed to copulate again (once as a female and once as a male) it laid two egg masses. The first, a small inviable one, was laid within 24 hours of copulation and a large viable egg mass was laid 24 hours later. No further eggs were laid although the individual was maintained in the laboratory for another month (Leonard, unpub. data). In one case, a *Navanax* laid a fertile egg mass after 32 days of isolation (Leonard and Lukowiak, 1985). In *Navanax* both the frequency and size of egg masses vary, and this could reflect size of the animal, food availability and/or sperm stores (Leonard, unpub. data). In isolated *Hermissenda crassicornis* (Escholtz), an aeolid nudibranch, Rutowski (1983) reported that some individuals produced as many as eight egg masses that were at least partially fertile. He reported that *Hermissenda* isolated upon collection produced an average of 3.7 ± 2.5 fertile egg masses within 24 days. He reported 20 days as the longest interval between fertile egg masses but did not indicate how long sperm can be stored after a copulation. In that study, sperm-deleted animals laid egg masses approximately three days after receiving an intromission. Another aeolid, *Phestilla melanobranchia* Bergh, can lay an average of 1.5 fertile egg masses per day for two weeks before depleting the sperm received in a single mating (Harris, 1975).

The possibility of self-fertilization complicates analysis of the relationship between egg laying and copulation in both basommatophoran and stylommatophoran pulmonates. In at least two species of the basommatophoran *Lymnaea*, egg laying begins sooner in mated than in isolated individuals (Horstmann, 1955; Boray, 1964; van Duivenboden, 1983, 1984). Horstmann (1955) established that this effect was mediated by the presence of allosperm in the gametolytic gland. However, van Duivenboden (1984) reported *Lymnaea* raised in groups laid fewer eggs than did isolated individuals.

NUMBER OF MATES

There are at least two reports of apparent monogamy in opisthobranchs. Lalli and Wells (1978: 103) concluded, from anatomical evidence, that in the pelagic pteropod *Limacina inflata* (d'Orbigny) a spermatophore is formed from the prostate gland which must be reciprocally transferred to a partner, because "all *L. inflata* of the proper size have either a well-developed prostate gland or a spermatophore received from another individual, but never both structures". Since this species is anatomically protandrous, an individual presumably mates only once in its lifetime. A different type of monogamy, involving iteroparity, has been reported from aeolid nudibranchs of the genus *Phestilla*. Rudman (1981: 408) reported that for two species from Tanzania, "At a very early stage individuals would pair with another individual of the same species and they would remain nestled together for their whole lifespan, except when egg-laying or feeding." Individual mature *Phestilla* deposit one or two egg masses per day for several weeks (Harris, 1975). However, as Hadfield and Switzer-Dunlap (1984) suggested, in most opisthobranch species individuals probably mate with a number of different individuals over their lifespan.

Tompa (1984) considered that multiple mating is probably the rule for stylommatophorans and cited evidence from *Cepaea nemoralis* (L.) (Murray, 1964) that an average brood is sired by two individuals and that 10-20 spermatophores have been found in an individual. Lind (1988) concluded that sperm competition will often occur in *Helix pomatia* L. under field conditions. Multiple paternity has also been reported for basommatophorans (Mulvey and Vrijenhoek, 1981; Monteiro *et al.*, 1984; Rudolph and Bailey, 1985). In summary, the available information, while scanty, suggests that while various types of monogamy can occur, most euthyneuran gastropods mate with several sexual partners over their lives and that there is considerable potential for sperm competition and multiple paternity of egg masses. Furthermore, the ability to store sperm for long periods of time can create special problems relative to certainty of paternity. Studies of gastropod reproductive anatomy and physiology from the standpoint of sexual selection and/or sexual conflict acting through sperm competition may shed light on both the function of gastropod reproductive tracts and the evolutionary biology and speciation of gastropods.

PREDICTIONS FROM THEORY

As stated above, the Hermaphrodite's Dilemma model predicts that the mating systems of simultaneous hermaphrodites, including gastropods should a) be based on reciprocity and b) involve a detectable level of "cheating" in a favored role. In some euthyneuran gastropods, reciprocity in the mating system is obvious and here the novel prediction is that of "cheating" in a favored role. In other groups reciprocity is not obvious or not known and thus is, in and of itself, a strong prediction of the model. A further problem is to predict which sexual role will be preferred. Bateman's principle and the egg-trading model of hermaphrodite mating systems (Charnov, 1979; Fischer, 1980; see also discussion in Leonard and Lukowiak, 1991) predict that the male role will be preferred except where the male contributes something other than sperm to the mating (i.e. parental care, a nutritional investment, etc.) which makes male parental investment larger than female parental investment. Because one cannot determine which investment is larger *a priori*, I assume here, for the sake of simplicity, that the egg-trading model consistently predicts a preference for the male role. The gamete-trading model (Leonard and Lukowiak, 1984, 1985) predicts that where the female controls fertilization the mating system will be based on sperm trading. That is, the female role will be preferred in general in euthyneuran gastropods, and that exceptions should be found in those species that lack a gametolytic gland and/or sperm storage. Thus, for most species of euthyneuran gastropod the egg-trading model predicts a preference for the male role and the gamete-trading model predicts a preference for the female roles. Specific predictions and tests of these models are discussed below.

CASE STUDIES OF MATING SYSTEMS

NAVANAX INERMIS AND SPERM-TRADING

There has been some progress in the analysis of sexual conflict in one of the unilaterally copulating opisthobranchs, *Navanax inermis* (Leonard and Lukowiak, 1984, 1985, 1987a, 1991), which has served as the stimulus for development of the gamete-trading and Hermaphrodite's Dilemma models. The mating system of *Navanax* is based on reciprocation in that pairs of individuals actively alternate sexual roles over a series of copulations. This mating system is analogous in many respects to that of the egg-trading serranid fishes (Leonard and Lukowiak, 1984, 1985; Leonard, unpub. data), in that two individuals remain together for a series of copulations but there is no long-term pair bond. The major difference is that in serranids the male role is preferred as predicted by Bateman's principle (Fischer, 1980; Leonard, unpub. data). In *Navanax*, both qualitative observations (Leonard and Lukowiak, 1984, 1985) and experimental tests (Leonard and Lukowiak, 1991) indicate that it is female, rather than the male, sexual role that is preferred. The preference for the female sexual role in *Navanax* has been hypothesized to be a consequence of female control of fertilization (Leonard and Lukowiak, 1984, 1985, 1991).

In *Navanax*, as in most euthyneuran gastropods (Pruvot-Fol, 1961; Tompa, 1984; Geraerts and Joosse, 1984; Hadfield and Switzer-Dunlap, 1984), there is both a sperm storage organ and a gametolytic gland (Rudman, 1974), and ovulation is not tied to copulation. Consequently, sperm transferred to a partner could be "wasted" in that they could be digested rather than stored, and if stored they could or could not be used for fertilization while they (the sperm) are still viable. In the serranids, on the other hand, female courtship displays are closely tied to ovulation and the "male" has reliable information as to the onset and duration of spawning (Fischer, 1980). Therefore, the "male" has greater control over the fate of its gametes than does the "female," and this could be generally true of externally-fertilizing fishes (Alexander and Borgia, 1979). The gamete-trading model predicts that simultaneous hermaphrodites will prefer the sexual role that offers control of fertilization (Leonard and Lukowiak, 1984, 1985, 1991). Therefore, the female role should be preferred in most euthyneuran gastropods (Leonard and Lukowiak, 1985).

The mating system of *Navanax*, termed sperm-trading, represents a mirror image of the egg-trading system. In *Navanax* a sexual encounter is initiated by an individual tracking down, courting and copulating in the male role (indicating willingness to reciprocate by starting out in the less-preferred sexual role). The available evidence suggests that males maintain intromission until the partner reciprocates (Leonard and Lukowiak, 1984, 1985, 1987a, 1991). This could serve to enforce reciprocation by preventing the partner from mating again as a female before serving as a male to its current partner. The (indirect) evidence that "cheating" occurs in *Navanax* is twofold: 1) reciprocation does not always occur, and 2) the complexity and variability of behavior observed during alternation of sexual roles suggests that cheating attempts may be occurring at this time (Leonard and Lukowiak, 1985, 1987a). In *Navanax*, sperm transfer is not directly observable and at present there is no evidence as to whether or not "subtle cheating" (Trivers, 1971) in the form of failures to transfer sperm during a copulation, or the transfer of substandard quantities of sperm, is occurring.

BIOMPHALARIA AND SPERM-SHARING

Evidence for what could represent a type of "subtle," probably even "victimless" cheating on a sperm-trading mating system, does exist in planorbid basommatophoran pulmonates of the genus *Biomphalaria*. Using genetic markers, Monteiro *et al.* (1984) demonstrated that *Biomphalaria* copulating as males sometimes transfer, to a female partner, sperm that they have received from a previous partner. That is, a snail copulating as a male may inseminate its partner with allosperm instead of, or along with, autosperm. This phenomenon appeared paradoxical at first glance because Bateman's principle can not explain how an individual would benefit by distributing someone else's sperm. The most probable functional explanation of this phenomenon is that it is a form of "cheating" in a sperm-trading mating system, serving to allow a snail to receive more sperm from a partner than it gives up (in autosperm) (Leonard and Lukowiak, 1987b; Monteiro *et al.*, 1987). This prediction was made on the basis of the gamete-trading model and analogy with *Navanax* (Leonard and Lukowiak, 1985, 1987b). This is a strong prediction because although copulation in *Biomphalaria glabrata* (Say), at least, is typically unilateral and reciprocation can occur (Brenner, 1990), the details of the mating system are as yet unknown. That is, it remains to be shown that the mating system is based on reciprocation with courtship performed by the male, and a preference for the female role, as is required for sperm-trading. Sperm-parcelling, in which only a small quantity of sperm (too little to fill the sperm storage organ) is transferred in a single copulation, is expected to form part of sperm-trading mating systems (Leonard and Lukowiak, 1984, 1985). M.E.B. Valadares-Ribeiro has obtained evidence from studies with genetic markers of sperm-parcelling in *B. tenajophila* (pers. comm. from W. Monteiro). Sperm-parcelling has yet to be demonstrated directly for *Navanax*, although there is some evidence that a single copulation as a female does not completely replenish depleted sperm stores (Leonard, unpub. data, see also above).

OTHER MATING SYSTEMS WITH UNILATERAL COPULATION

To date, *Navanax* is the only species for which repeated alternation of sexual roles in a copulatory bout has been described. Further investigation could show that this is not uncommon in unilaterally copulating gastropods since very little is known about the copulatory behavior of most of them. However, it is clear that repeated alternation is not characteristic of some taxa, e.g. *Lymnaea, Aplysia*, etc. There are three types of mating interaction that have been described for these species: single non-reciprocal copulations, a single alternation of sexual roles, and chain copulation.

LYMNAEA AND OTHER BASOMMATOPHORANS

A single alternation of sexual roles commonly occurs in the lymnaeid basommatophorans, *Lymnaea stagnalis* (L.) (Noland and Carriker, 1946; Barraud, 1957; van Duivenboden, 1984) and *Stagnicola elodes* (Say) (Rudolph, 1979a) and perhaps also in the stylommatophoran genus *Partula* (Lipton and Murray, 1979). In contrast, reciprocation does not occur commonly in the planorbid basommatophoran *Bulinus globosus* (Morelet) (Rudolph, 1979b). The mechanisms by which reciprocation is enforced (or cheating prevented) in cases of a single alternation of sexual roles have not been studied in detail. In *S. elodes* and *L. stagnalis*, male sexual behavior has been shown to be induced by copulation as a female (Rudolph, 1979a; v. Duivenboden and ter Maat, 1985) and this could also be the case in *Navanax* (Leonard and Lukowiak, 1991). In *Lymnaea*, courtship is a male behavior and individuals appear to be always willing to copulate as females (van Duivenboden and ter Maat, 1985), which suggests that the female sexual role is preferred. Also, copulatory plugs have been reported in *S. elodes* and *B. globosus* (Rudolph, 1979a, b). That of *S. elodes* probably prevents a second copulation as a female for two to three hours, while in *B. globosus* the copulatory plug is presumed to be ineffective in preventing a second copulation. It would be interesting to know more about the relationship of these plugs, apparently common in basommatophorans (Geraerts and Joosse, 1984), to the mating system.

These instances of apparently unilateral and/or single reciprocal copulations offer exciting opportunities to test the Hermaphrodite's Dilemma model, because they appear at first glance to contradict the predictions of the model. The first prediction of the model is that of reciprocity. Thus in order to be consistent with the model, the cases of unilateral copulation must actually represent part of a reciprocal interaction [which is not impossible, the single alternation of *Lymnaea stagnalis* could involve a period of hours between the two copulations during which the first male rides on the shell of the first female before she begins to reciprocate (Leonard, v. Duivenboden and ter Maat, unpub. data)] or the unilateral copulations must represent a form of "cheating" obviously derived from a reciprocal mating system. The cases of a single reciprocal copulation are also puzzling. Single reciprocal copulations would be consistent with the predictions of the Hermaphrodite's Dilemma only under Game of Chicken conditions. That is, if there is a preferred role, an individual that has taken that role in the first copulation would have no reason to remain and reciprocate by assuming the less preferred role, unless there were a shortage of other mates available. Because many of the basommatophorans, such as *Lymnaea* and *Physa*, for example, typically occur in dense populations this seems unlikely. Such population densities suggest that Prisoner's Dilemma conditions should be in operation but it is axiomatic (Axelrod and Hamilton, 1981) that under Prisoner's Dilemma, reciprocation (such as Tit-for-Tat) can only evolve where the last move of the interaction is known. Otherwise, each player would benefit by refusing to reciprocate on the last move (see Leonard, 1990). Therefore, the Hermaphrodite's Dilemma model predicts that single reciprocal copulations must involve as yet identified mechanisms for enforcing reciprocation. Specifically, where a gametolytic gland exists, it should be the case that the male is able to prevent the female from leaving before reciprocating by assuming the male role with its former partner. The egg-trading model, on the other hand, would predict that individuals should compete for opportunities to copulate as males.

CHAIN COPULATION

Chain copulation consists of a mating interaction between three or more individuals in which the individual in front acts only as a female, while each middle individual acts both as a male (to the individual in front of it) and as a female (to the individual behind it) while the last individual acts only as a male. This phenomenon has been observed in a variety of euthyneuran gastropods, particularly basommatophoran pulmonates and tectibranch opisthobranchs (see various planorbids, Precht, 1936; Duncan, 1975; Kuma, 1975; Geraerts and Joosse, 1984; Hadfield and Switzer-Dunlap, 1984; Franc, 1986). Chain (and even ring) formation appears possible in virtually all species that normally copulate unilaterally, and some that usually copulate reciprocally (e.g. *Phyllaplysia taylori* Dall, Beeman, 1970a, b), and is particularly common under crowded laboratory conditions. In some taxa, such as *Aplysia* spp., however, chain copulation appears to occur commonly in the field, and must be regarded as a normal feature of the mating system (P. Fischer, 1869; MacGinitie and MacGinitie, 1968; Ricketts *et al.*, 1968; Kupfermann and Carew, 1974; Leonard, unpub. data). Chain copulation has also been described from laboratory observations for *Acera bullata* Müller (Legendre, 1905). Geldiay (1956) concluded that chain copulation was the rule rather than the exception for Lake District populations of the freshwater limpet, *Ancylus fluviatilis* Müller, where chains of as many as seven individuals have been observed. Wesenberg-Lund (1939) reported for *Lymnaea* that chains of three individuals were not uncommon in the field (see also Crabb, 1927; Noland and Carriker, 1946; Barraud, 1957; v. Duivenboden, 1984) and that the female will next act as male to a nearby individual. There are also reports of simultaneous reciprocal copulation in *Lymnaea* (Klotz, 1889; Crabb, 1927). In other species, chain copulation is probably largely an artifact of laboratory conditions [e.g. *Physa fontinalis* (L.) (Duncan, 1959)] and rare, if it occurs at all, in the field. For example, in *Navanax* chains and/or rings of three or four copulating individuals occur commonly in the laboratory, but are very rare in the field (Leonard and Lukowiak, 1985). Rivest (1984) described group hypodermic copulation in two species of the nudibranch *Palio*, but this appears to be the exception, the rule being simultaneously reciprocal hypodermic copulation.

The Hermaphrodite's Dilemma model predicts that chain copulation should represent an obvious derivative from a reciprocal mating system. The data available in the literature are not adequate to confirm or refute this prediction and further observations are required before we can understand chain copulation as a mating system. In *Lymnaea*, mating interactions typically involve a single alternation of sexual roles between members of a pair (Noland and Carriker, 1946; Barraud, 1957; v. Duivenboden, 1984; v. Duivenboden and ter Maat, 1985; Leonard, v. Duivenboden and ter Maat, unpub. obs.) and it could be the case that chain copulations occur under conditions of high density and could represent "cheating" on a successively reciprocal system. If so, the gamete-trading model predicts that the "cheating" will consist of females avoiding male behavior, whereas the egg-trading model would predict that individuals should compete for opportunities to copulate as males. Another possibility is that mechanisms exist for reciprocation within the chain interaction. That is, individuals in chains could remain in the chain until they have copulated equally often in both roles. Some observations in both *Lymnaea* (Wesenberg-Lund, 1939) and *Aplysia californica* (Leonard and Lukowiak, 1983; Leonard, unpub. data) suggest that, as in *Navanax* (Leonard and Lukowiak, 1987a, 1991), individuals begin to act as males after acting as females. Also, laboratory observations indicate that chains of copulating *A. californica* can break and reform and individuals can copulate several times before mating activity ceases, with some indication that females (individuals at the front of the chain) tend to act as males to either the animal at the end of the chain or a nearby individual in the subsequent copulation (Leonard and Lukowiak, unpub. data). The data, however, are too scanty to allow us to tell whether individuals alternate sexual roles within chains. In *Aplysia*, courtship is initiated by the individual that will act as a male (Kupfermann and Carew, 1974; Leonard and Lukowiak, 1983) as is the case in *Lymnaea* and *Navanax* but there is as yet no clear evidence that the female is preferred as predicted by the gamete-trading model.

THE STRANGE CASE OF *ARIOLIMAX*: SELF-MULTILATION? HERMAPHRODITES AS "CASTRATING FEMALES"?

Another intriguing observation is the report for a stylommatophoran slug, *Ariolimax*, that "they frequently gnaw off the penis at the close of copulation," (Mead 1943: 675). A certain percentage of large individuals in *Ariolimax* appear to lack completely a penis (Heath, 1916; Mead, 1943), whereas in others it is underdeveloped (Heath, 1916). Heath (1916), having hypothesized that the penis must be lost and then regenerated in this species, collected 200 individuals in an enclosure and after several weeks was able to observe two instances of copulation. He described the courtship process and stated that copulation was unilateral and that in both cases the penis was chewed off as soon as the animals began to draw apart. He indicated that in at least one case the amputation was initiated by the female who was then joined in amputation by the "possessor of the intromittent organ concerned". Upon dissection Heath found that in two of the individuals (the females in the copulations), the amputated penis extended from the genital pore internally to the distal end of the seminal receptacle. Heath found this phenomenon understandably perplexing and offered two possible explanations: 1) that the amputated penis serves as a sperm plug; 2) that the behavior is an artifact of disturbance by the observer and has evolved as a means of rapid separation when escape is necessary. Because the amputation process took over 10 min (Heath, 1916), it seems unlikely that it is an effective defense against predators but it could serve to prevent dessication. Heath mentioned that copulation is nocturnal and that intromission had lasted several hours before the animals began to separate so it may be the case that copulations starting late in the night might create a risk of dessication in the morning sun unless there was a way of rapidly terminating them. Sexual conflict theory can add 1) the possibility that the function of the sperm plug is more to prevent the mate from acting as a female again (keep other sperm out) than to prevent loss of sperm, and/or 2) the suggestion that in these hermaphrodites an individual that amputated the penis of its mate could increase its own reproductive success as a male by decreasing the number of effective male rivals.

The Hermaphrodite's Dilemma model would predict that this behavior, if it is not merely a defense mechanism against danger of dessication or the like, must be a means of enforcing reciprocation or "cheating" on a reciprocal mating system. For example, if as predicted by the gamete-trading model, the female role is preferred it could be the case that once an individual has accumulated enough allosperm to fill its own sperm storage organ, it could not have any "reason" to mate as a male and could amputate its own penis, leaving it as a sperm plug to prevent its mate from receiving more sperm before egg-laying, thereby insuring paternity. It is barely conceivable that an individual could be able to regenerate its penis in time to get to use it in reciprocal mating interactions in order to obtain a new load of sperm after using the previous batch. In any case, the gamete-trading model predicts that *Ariolimax* which lack a penis should remain willing to copulate as females whereas the egg-trading model would predict that, because hermaphrodites should copulate as females in order to get an opportunity to copulate as males, that an individual lacking a penis ought to be unwilling to copulate as a female. Similarly, if the goal of copulating as a male is getting an opportunity to be female, as is predicted for euthyneuran gastropods by the gamete-trading hypothesis, then *Ariolimax* should be reluctant to act as a male to an individual that lacks a penis. The egg-trading model on the other hand predicts that an individual copulating as a male should not be fussy and should accept a mate with or without a penis of its own. Therefore, both gamete-trading and egg-trading predict that individuals lacking a penis will be unlikely to be involved in copulations but the egg-trading model predicts that that will be due to "coyness" of the amputee, whereas the gamete-trading model predicts that the individual lacking a penis will be unattractive or rejected as a (female) mate.

The copulation of these common banana slugs of the northwest coast of the United States seems to demand further attention. We need to know: 1) whether this amputation is a defensive response or whether it occurs as a normal part of the sexual behavior; 2) how commonly this amputation occurs; 3) who amputates the penis of whom; and 4) whether this amputation occurs after an individual's first copulation or only in older individuals who may have mated with several partners. We also need to know how often these animals copulate over their life-span, and/or between egg-layings in order to understand the significance of this. Perhaps these slugs are effectively monogamous, at least as males, each individual mating once upon attaining adulthood and losing its penis in the process, with occasional individuals surviving long enough to regenerate the penis and copulate as males a second time? Its amazing how little we know about such common and conspicuous animals.

SIMULTANEOUSLY RECIPROCAL COPULATION

STYLOMMATOPHORANS

Both Hyman (1967) and Franc (1968) made the interesting generalization that basommatophorans have unilateral copulation associated with a short, simple courtship performed by the individual that will act as the male, whereas stylommatophorans have simultaneously reciprocal copulation preceded by lengthy, elaborate, and often bizarre courtship behavior. The usual explanation of this phenomenon has been mechanistic; i.e. that the behavior serves to facilitate coordination between the partners to allow simultaneous reciprocal intromission, and most of the experimental work has focused on that aspect of the behavior (i.e. *Helix*, Jeppesen, 1976; Lind, 1976; Chung, 1986; Adamo and Chase, 1988; Giusti and Andreini, 1988). However, because many opisthobranchs, particularly nudibranchs, have simultaneous reciprocal copulation without lengthy or notably peculiar courtship behavior (Hadfield and Switzer-Dunlap, 1984; Leonard, unpub. data; see also aeolids below), it is difficult to argue that simultaneous reciprocal copulation must be accompanied in evolution by such bizarre mechanisms as the love-dart of *Helix*, the ingestion of caudal mucous globules, the aerial performance of *Limax maximus* (L.), etc. (see Hyman, 1967; Franc, 1968; Tompa, 1980). An obvious functional or adaptive explanation is that these elaborate courtship behaviors have evolved through sexual conflict.

Specifically, the Hermaphrodite's Dilemma model predicts that they all serve to prevent "cheating" on the reciprocal mating system, which should take (according to the gamete-trading model) the form of individuals attempting to act as females, receiving sperm, without offering any of their own. Both Meisenheimer (1907) and Lind (1976) reported that in *Helix*, that an individual (A) that inserts its penis into the vagina of its partner (B) will immediately withdraw its penis unless B simultaneously inserts its (B's) penis into A's vagina (but see Chung, 1987). This is consistent with the idea that courtship serves to prevent an individual from acting only as a male. Similar reluctance to act as a male before the partner does should be seen in other species with elaborate courtship and simultaneous reciprocal copulation. The courtship therefore, should enforce reciprocity, specifically by preventing individuals from acting only as females. An egg-trading model, based on Bateman's principle would predict the opposite; that is the courtship serves to enforce reciprocity by preventing individuals from "cheating" by acting only in the male role. One would predict therefore that in these elaborate courtships there should be evidence that individuals are 1) coy as females, refusing to allow intromission until they have an opportunity to act as a male; and 2) eager as males, competing with each other for the first intromission. Charnov (1979) suggested that such "complicated precopulatory displays" should serve (under Bateman's principle) to induce the partner to use the sperm received to fertilize eggs. These hypotheses should be testable by experimental and comparative studies of common species of stylommatophorans.

AEOLIDS

While Lind (1976) reported that unilateral copulations were rare in *Helix*, Rutowski (1983) found that 49% of all copulations in *Hermissenda* were non-reciprocal. Rutowski (1983) discussed this phenomenon in terms of sexual conflict and Charnov's (1979) prediction, considering the possibility that the failure of one individual to intromit after everting its penis was the result of an effort by its partner either to deflect the penis of its partner or to give sperm quickly without receiving any. That is, that one individual was attempting to "cheat" by mating only in the male role. He concluded that this was unlikely because many of the "missed individuals" were sperm-depleted and would have benefited from receiving sperm.

If, as the gamete-trading model (Leonard and Lukowiak, 1984, 1985) predicts, the female sexual role is preferred, the "cheater" in a unilateral copulation would be the individual receiving but not giving sperm. These unilateral intromissions in *Hermissenda* could represent "cheating" by "deliberately" missing the target. However, since Rutowski (1983) reported that sperm was ejaculated into the water as a result of these "missed" intromissions this seems improbable. It seems very unlikely that emission of sperm into the water would be more adaptive than transferring it to a partner, especially because Rutowski's (1985) observation that *Hermissenda* ingest any sperm left on the gonopore suggests that the caloric content of sperm is not trivial. I agree, therefore, with Rutowski's conclusion that the high frequency (49%) of copulations in which only one individual achieves intromission is probably a consequence of whatever factors have selected for extremely rapid copulation in this species. However, on the assumption that the female sexual role is preferred in *Hermissenda*, I suggest that cheating was represented in Rutowski's observations by those copulations (5% of the total) which were unilateral because only one individual everted its penis, because in these cases individuals received sperm without giving any in return (or wasting any). A more detailed study of the mating behavior of *Hermissenda* as a function of the sperm stores of the interacting individuals might serve to test this possibility. In particular, Rutowski's observation that most of the "missed individuals" in only semi-successful reciprocal copulation attempts were sperm-depleted is intriguing. One would like to know if sperm-depleted animals behave differently during mating encounters, and/or if their depleted status is detectable by partners who then treat them differently. One would expect that, if there is any difference, sperm-depleted individuals ought to be both more willing to receive sperm, and more attractive as female partners, than individuals with full sperm stores.

Observations from another aeolid, *Aeolidia papillosa* Bergh, are also suggestive of cheating in a mating system based on reciprocation. The sexual behavior of *Aeolidia* is very similar to that of *Hermissenda* (Longley and Longley, 1982, 1984); encounters are very brief and usually simultaneously reciprocal. However, in *Aeolidia* there is no copulation, sperm packets are deposited on the partner's gonopore (Longley and Longley, 1984). The Longleys observed one individual which copulated repeatedly (over a period of days) without producing sperm packets. These authors also reported that the quantity of sperm transferred in a copulation was determined by both 1) the duration of the copulation, which is correlated with the size of the smaller partner, and 2) the rate of sperm transfer, which is related to the number of autosperm remaining in the ampulla. This raises the possibility that *Aeolidia* could engage in what Trivers (1971) termed "subtle cheating." That is, an *Aeolidia* could cheat by engaging in a reciprocal mating when it has relatively few autosperms available, and thereby receive more sperm than it gives to its partner. Beeman (1970a) also observed instances in which only one member of a pair of reciprocally copulating *Phyllaplysia taylori* Dall transferred sperm to its partner, since the other's ampulla was empty.

Observations on many species with simultaneous reciprocal copulation mention that unilateral copulations sometimes occur (e.g. *Helix*: Herzberg and Herzberg, 1962; Lind, 1976; opisthobranchs: Hadfield and Switzer-Dunlap, 1984; including *Aeolidia papillosa*: Longley and Longley, 1984; *Hermissenda crassicornis*: Longley and Longley, 1982; Rutowski, 1983; *Melilbe*: Agersborg, 1922) which could be considered "cheating." In summary, there is some evidence that "cheating" can occur occasionally in species with simultaneous reciprocal copulation, in the form of unilateral copulations and/or "subtle cheating." However, from the available evidence one cannot say with confidence that "cheating" does or does not occur in these species. The evidence does suggest that studies directed to the analysis of sexual conflict in this group would be very rewarding.

DISCUSSION

The review and analysis presented here suggest that 1) gastropods offer a broad array of reproductive phenomena that require explanation in terms of mating systems theory; 2) Hermaphrodite's Dilemma model makes nontrivial predictions about the mating systems of hermaphroditic gastropods that may serve to test the model; 3) for many euthyneuran gastropods the egg-trading and gamete-trading models make opposing predictions, making this group a useful means of distinguishing between the two models. While experimental studies can be used to determine the preferred sexual role for a given species (Leonard and Lukowiak, 1991), comparative studies can also be useful here. Review of the range of mating systems found in simultaneously hermaphroditic serranid fishes (Leonard, unpub. data) provides strong support for the existence of sexual conflict since it provides confirmation of the hypothesis that the male sexual role is preferred in these simultaneous hermaphrodites, as assumed by Fischer (1980, 1984) and predicted by Charnov (1979). The evidence for this is twofold. First, in all species studied, ''cheating,'' on a reciprocal mating system, whether as streaking or as extra-pair spawning in the monogamous *Serranus tigrinus* (Bloch) (Pressley, 1981), is a male behavior; cheaters ''cheat'' in order to fertilize someone else's eggs, not to get their own eggs fertilized. Second, where mates become a defensible resource, large, dominant individuals become male (e.g. *S. fasciatus* (Jenyns) and *S. baldwini* (Evermann and Marsh) (Petersen, 1990). In serranids, then, the harem-based mating systems are exceptions that prove the egg-trading rule. These simultaneously hermaphroditic fish provide strong evidence that sexual conflict both exists and is important in shaping mating systems. However, both the gamete-trading and the egg-trading models predict (indeed the gamete-trading model assumes) that serranids prefer the male role so that the serranids do not allow us to distinguish between the egg-trading and gamete-trading models (contrary to Fischer, 1987). The gastropods therefore, offer an exciting opportunity not only to test the assumption that sexual conflict exists but also to distinguish between models based on different assumptions about the source of sexual conflict. The variety of reproductive behavior and physiology found within the gastropods should allow us to identify species that can be used to test hypotheses about the relative importance of energy investment, mating time and control of fertilization in sexual conflict.

In this paper I have attempted to demonstrate that analysis in terms of sexual conflict makes specific predictions about gastropod mating systems that may allow us to elucidate the adaptive significance of many bizarre phenomena in gastropod reproductive biology. The available literature on gastropod sexual behavior suggests a number of interesting test cases for the Hermaphrodite's Dilemma model, but does not, in itself, provide sufficient data to test the model. The chief difficulty interpreting the available information on gastropod reproduction in terms of sexual conflict or sexual selection is that one can seldom determine from the available descriptions which types of behavior represent the rule and which the exceptions. Where the initial studies were not informed by mating systems theory or selection thinking, crucial information is apt to be lacking, even when there have been numerous detailed studies of the behavior, as in *Helix* (Leonard, unpub. data). Mating systems theory has a lot to offer to the study of euthyneuran gastropods and euthyneuran gastropods have a lot to offer to the study of mating systems theory.

ACKNOWLEDGMENTS

The author was supported by grants from the NIMH and NSF during part of the period of manuscript preparation.

LITERATURE CITED

Adamo, S. A. and R. Chase. 1988. Courtship and copulation in the terrestrial snail *Helix aspersa. Canadian Journal of Zoology* 66:1446-1453.

Agersborg, H. P. v. W. Kjerschow. 1921. Contribution to the knowledge of the nudibranchiate mollusk, *Melibe leonina* (Gould). *American Naturalist* 55:222-253.

Alexander, R. D. and G. Borgia. 1979. On the origin and basis of the male-female phenomenon. *In: Sexual selection and reproductive competition in insects.* M. S. Blum and N. A. Blum, eds. pp. 417-440. Academic Press, Inc., New York.

Axelrod, R. and W. D. Hamilton. 1981. The evolution of cooperation. *Science* 211:1390-1396.

Barraud, E. M. 1957. The copulatory behavior of the freshwater snail (*Lymnaea stagnalis* L.). *British Journal of Animal Behaviour* 5:55-59.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila. Heredity* 2:349-368.

Baylis, J. R. 1981. The evolution of parental care in fishes, with reference to Darwin's rule of male sexual selection. *Environmental Biology of Fishes* 6:223-251.

Beeman, R. D. 1970a. The anatomy and functional morphology of the reproductive system in the opisthobranch mollusk, *Phyllaplysia taylori* Dall, 1900. *Veliger* 13:1-31.

Beeman, R. D. 1970b. An ecological study of *Phyllaplysia taylori* Dall, 1900 Gastropoda: Opisthobranchia with an emphasis on its reproduction. *Vie et Milieu* Sec. A 21:189-211.

Berglund, A. 1986. Sex change by a polychaete: Effects of social and reproductive costs. *Ecology* 67:837-845.

Boray, J. C. 1964. Studies on the ecology of *Lymnaea tomentosa,* the intermediate host of *Fasciola hepatica.* II. The sexual behaviour of *Lymnaea tomentosa. Australian Journal of Zoology* 12:231-237.

Brenner, V. A. 1990. The effect of isolation on the reproductive behavior and fecundity of *Biomphalaria glabrata* (Mollusca: Pulmonata). Undergraduate Honors Thesis, Department of Zoology, University of Oklahoma, Norman. 11 pp.

Bronstein, J. L. 1988. Limits to fruit production in a monoecious fig: consequences of an obligate mutualism. *Ecology* 69:207-214.

Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences, U.S.A.* 76:2480-2484.

Charnov, E. L. 1982. *The Theory of Sex Allocation.* Princeton University, Press, Princeton, New Jersey. 355 pp.

Charnov, E. L. 1986. Size advantage may not always favor sex change. *Journal of Theoretical Biology* 119:283-285.

Chung, D. J. D. 1986. Stimulation of genital eversion in the land snail *Helix aspersa* by extracts of the glands of the dart apparatus. *Journal of Experimental Zoology* 238:129-139.

Chung, D. J. D. 1987. Courtship and dart shooting behavior of the land snail *Helix aspersa. Veliger* 30:24-29.

Crabb, E. D. 1927. The fertilization process in the snail *Lymnaea stagnalis appressa* Say. *Biological Bulletin* 53:67-109.

Darwin, C. 1874. *The Descent of Man and Selection in Relation to Sex* (2nd ed.). Rand McNally and Co., Chicago. 672 pp.

Davis, M. D. 1983. *Game Theory.* Revised edition. Basic Book, Inc., New York. 250 pp.

Duncan, C. J. 1959. The life cycle and ecology of the freshwater snail *Physa fontinalis* (L.). *Journal of Animal Ecology* 28:97-117.

Duncan, C. J. 1975. Reproduction. *In: Pulmonates,* Vol. I. *Functional Anatomy and Physiology.* V. Fretter and J. Peake, eds. pp. 309-365. Academic Press, London.

Duivenboden, Y. A. van. 1983. Transfer of semen accelerates the onset of egg-laying in female copulants of the hermaphrodite freshwater snail *Lymnaea stagnalis. International Journal of Invertebrate Reproduction* 6:249-257.

Duivenboden, Y. A. van. 1984. Sexual behavior of the hermaphrodite freshwater snail *Lymnaea stagnalis.* Doctoral Dissertation. Vrije Universiteit, Amsterdam. 109 pp.

Duivenboden, Y. A. van and A. ter Maat. 1985. Masculinity and receptivity in the hermaphrodite snail, *Lymnaea stagnalis. Animal Behaviour* 33:885-891.

Eberhard, W. G. 1985. *Sexual Selection and Animal Genitalia.* Harvard University Press, Cambridge, Massachusetts. 244 pp.

Emlen, S. T. and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215-223.

Fischer, E. A. 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, *Hypoplectrus nigricans* (Serranidae). *Animal Behaviour* 28:620-633.

Fischer, E. A. 1984. Egg trading in the chalk bass, *Serranus tortugarum,* a simultaneous hermaphrodite. *Zeitschrift für Tierpsychologie* 66:143-151.

Fischer, E. A. 1987. Mating behavior in the black hamlet - gamete trading or egg trading? *Environmental Biology of Fishes* 18:143-148.

Fischer, E. A. and C. W. Petersen. 1987. The evolution of sexual patterns in the seabasses. *BioScience* 37:482-489.

Fischer, P. 1869. Sur l'accouplement et la ponte des Aplysiens. *Comptes Rendus de l'Academie des Sciences,* Paris 69:1095-1097.

Fisher, A. C. 1981. *Resource and Environmental Economics.* Cambridge University Press, Cambridge, England. 284 pp.

Fisher, R. A. 1958. The Genetical Theory of Natural Selection. Second revised edition. Dover Publications, Inc., New York. 291 pp.

Franc, A. 1968. Class des Gastéropodes (Gastropoda Cuvier, 1798). *In: Traité de Zoologie* Tome V, Fasc. 3. P.-P. Grasse, ed. pp. 1-925. Masson et Cie., Paris.

Galen, C. and Rotenberry, J. T. 1988. Variance in pollen carryover in animal-pollinated plants: Implications for mate choice. *Journal of Theoretical Biology.* 135:419-429.

Geldiay, R. 1956. Studies on local populations of the freshwater limpet *Ancylus fluviatilis* Müller. *Journal of Animal Ecology* 25:389-402.

Geraerts, W. P. M. and J. Joosse. 1984. Freshwater Snails (Basommatophora). *In: The Mollusca,* Vol. 7, Reproduction. A. S. Tompa, N. H. Verdonk and J. A. M. van der Biggelaar, eds. pp. 141-207. Academic Press, Orlando, Florida.

Gerhardt, U. 1933. Zur Kopulation der Limaciden. 1. Mitteilung. *Zeitschrift für Morphologie und Ökologie der Tiere* 27:401-450.

Ghiselin, M. T. 1965. Reproductive function and the phylogeny of opisthobranch gastropods. *Malacologia* 3:329-378.

Ghiselin, M. T. 1974. *The Economy of Nature and the Evolution of Sex.* University of California Press, Berkeley. 346 pp.

Gilbertson, L. H. 1989. A new species of *Holospira* (Gastropoda: Pulmonata) from Arizona, with the reproductive anatomies of *H. arizonensis* and *H. chiricahuana. Veliger* 32:308-312.

Gillespie, J. H. 1977. Natural selection for variances in offspring numbers: a new evolutionary principle. *American Naturalist* 111:1010-1014.

Giusti, F. and S. Andreini. 1988. Morphological and ethological aspects of mating in two species of the family Helicidae (Gastropoda Pulmonata): *Theba pisana* (Mueller) and *Helix aperta* Born. *Monitore Zoologica Italiano* (N.S.) 22:331-363.

Gosliner, T. M. 1980. Systematics and phylogeny of the Aglajidae (Opisthobranchia: Mollusca). *Zoological Journal of the Linnean Society* 68:325-360.

Hadfield, M. G. and M. Switzer-Dunlap. 1984. Opisthobranchs. *In: The Mollusca,* Vol. 7, Reproduction. A. S. Tompa, N. H. Verdonk and J. A. M. van der Biggelaar, eds. pp. 209-350. Academic Press, Orlando, Florida.

Hammerstein, P. and G. A. Parker. 1987. Sexual selection: games between the sexes. *In: Sexual Selection: Testing the Alternatives.* J. W. Bradbury and M. B.Andersson, eds. pp. 119-142. John Wiley and Sons.

Harris, L. G. 1975. Studies on the life history of two coral-eating nudibranchs of the genus *Phestilla. Biological Bulletin* 149:539-550.

Heath, H. 1916. The conjugation of *Ariolimax californicus. Nautilus* 30:22-24.

Herzberg, F. and A. Herzberg. 1962. Observations on reproduction in *Helix aspersa. American Midland Naturalist* 68:297-306.

Hoffmann, R. J. 1983. The mating system of the terrestrial slug *Deroceras laeve. Evolution* 37:423-425.

Horstmann, J. -J. 1955. Untersuchungen zur Physiologie der Begattung und Befruchtung der Schlammschnecke *Lymnaea stagnalis L. Zeitschrift für die Morphologie und Ökologie der Tiere.* 44:222-268.

Hyman, L. H. 1967. *The Invertebrates.* Vol. 6. *Mollusca I.* McGraw-Hill Book Co., New York. 792 pp.

Jeppesen, L. L. 1976. The control of mating behaviour in *Helix pomatia* L. (Gastropoda: Pulmonata). *Animal Behaviour* 24:275-290.

Klotz, J. 1889 . Beitraege zur Entwicklungsgeschichte und Anatomie des Geschlechtsapparate von *Lymnaeus. Jenaische Zeitschrift für Naturwissenschaften* 23:1-40.

Kuma, E. 1975. Studies on the behaviour of *Bulinus (Physopsis) globosus* (Morelet). *Zoologischer Anzeiger* 194:6-12.

Kupfermann, I. and T. J. Carew. 1974. Behavior patterns of *Aplysia californica* in its natural environment. *Behavioral Biology* 12:317-337.

Lalli, C. M. and F. E. Wells. 1978. Reproduction in the genus *Limacina* (Opisthobranchia: Thecosomata). *Journal of Zoology* 186:95-108.

Legendre, R. 1905. Notes biologiques sur *Acera bullata* Mull. *Archives de zoologie experimentelle et generale,* Ser. 4, Vol. 4:vi-xiv.

Leonard, J. L. 1990. The Hermaphrodite's Dilemma. *Journal of Theoretical Biology* 147:361-372.

Leonard, J. L. and K. Lukowiak. 1983. The behavior of *Aplysia californica:* Ethogram and ethological analysis of mating. *Society for Neuroscience Abstracts* 9:534.

Leonard, J. L. and K. Lukowiak. 1984. Male-female conflict in a simultaneous hermaphrodite resolved by sperm-trading. *American Naturalist* 124:282-286.

Leonard, J. L. and K. Lukowiak. 1985. Courtship, copulation and sperm trading in the sea slug, *Navanax inermis* (Opisthobranchia: Cephalaspidea). *Canadian Journal of Zoology* 63:2719-2729.

Leonard, J. L. and K. Lukowiak. 1987a. Ethological mechanisms of sexual role alternation in *Navanax inermis* (Opisthobranchia). *In: Neurobiology, Molluscan Models.* H. H. Boer, W. P. M. Geraerts and J. Joosse, eds. pp. 242-247. Monographie Koninklijke Nederlandse Akademic. van Wetenschappen, North Holland Publ. Co., Amsterdam.

Leonard, J. L. and K. Lukowiak. 1987b. Sperm-sharing in *Biomphalaria* snails. *Nature* 325:737-738.

Leonard, J. L. and K. Lukowiak. 1991. Sex and the simultaneous hermaphrodite: testing models of male-female conflict in a sea slug. *Animal Behaviour* 41:255-266.

Lind, H. 1976. Casual and functional organization of the mating behaviour sequence in *Helix pomatia* (Pulmonata, Gastropoda). *Behaviour* 59:162-202.

Lind, H. 1988. The behaviour of *Helix pomatia* L. (Gastropoda, Pulmonata) in a natural habitat. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening.* 147:67-92.

Lipton, C. S. and J. Murray. 1979. Courtship of land snails of the genus *Partula. Malacologia* 19:129-146.

Longley, R. D. and A. J. Longley. 1982. *Hermissenda*: Agonistic behavior or mating behavior? *Veliger* 24:230-231.

Longley, A. J. and R. D. Longley. 1984. Mating in gastropod mollusk *Aeolidia papillosa:* behavior and anatomy. *Canadian Journal of Zoology* 62:8-14.

Luce, R. D. and H. Raiffa. 1957. *Games and Decisions.* John Wiley and Sons, New York. 509 pp.

MacGinitie, G. E. 1934. The egg-laying activities of the sea hare, *Tethys californicus* (Cooper). *Biological Bulletin* 67:300-303.

MacGinitie, G. E. and N. MacGinitie. 1968. Natural History of Marine Animals (2nd ed.). McGraw-Hill Book Company, New York. 523 pp.

Mead, A. R. 1943. Revision of the giant West Coast land slugs of the genus *Ariolimax* Moerch (Pulmonata: Arionidae). *American Midland Naturalist* 30:675-717.

Meisenheimer, J. 1907. Biologie, Morphologie und Physiologie des Begattungsvorgangs und der Eiablage von *Helix pomatia. Zoologische Jahrbücher, Abteilung für Systematik* 25:461-502.

Monteiro, W., J. M. G. Almeida, Jr. and B. S. Dias. 1984. Sperm-sharing in *Biomphalaria* snails: a new behavioral strategy in simultaneous hermaphroditism. *Nature* 308:727-279.

Monteiro, W., J. M. G. Almeida, Jr. and B. S. Dias. 1987. Monteiro *et al.* reply. *Nature* 325:737-738.

Mulvey, M. and R. C. Vrijenhoek. 1981. Multiple paternity in the hermaphroditic snail, *Biomphalaria obstructa. Journal of Heredity* 72:308-312.

Murray, J. 1964. Multiple mating and effective population size in *Cepaea nemoralis. Evolution* 18:283-291.

Nakamura, R. R., M. L. Stanton and S. J. Mazer. 1989. Effects of mate size and mate number on male reproductive success in plants. *Ecology* 70:71-76.

Nicholson, W. 1978. *Microeconomic Theory.* Dryden Press, Hinsdale, Illinois 768 pp.

Noland, L. E. and M. R. Carriker. 1946. Observations on the biology of the snail *Lymnaea stagnalis appressa* during twenty generations in laboratory culture. *American Midland Naturalist* 36:467-493.

Orians, G. H. 1969. On the evolution of mating systems in birds and animals. *American Naturalist* 103:589-603.

Parker, G. A. 1979. Sexual selection and sexual conflict. *In: Sexual Selection and Reproductive Competition in Insects.* M. S. Blum and N. A. Blum, eds. pp. 123-166. Academic Press, New York.

Patterson, C. M. 1989. Morphological studies of a Tahitian succineid, *Succinea (Kondosuccinea) wallisi. Malacological Review* 22:17-23.

Pelseneer, P. 1895. Hermaphroditism in Mollusca. *Quarterly Journal of Microscopical Science* 37:19-46.

Petersen, C. W. 1990. The relationships among population density, individual size, mating tactics, and reproductive success in a hermaphroditic fish, *Serranus fasciatus. Behaviour* 113:57-80.

Philippi, T. and J. Seger. 1989. Hedging one's evolutionary bets, revisited. *Trends in Ecology and Evolution* 4:41-44.

Precht, H. 1936. Zur Kopulation und Eiablage einiger Planorbiden. *Zoologischer Anzeiger* 115:80-89.

Pressley, P. H. 1981. Pair formation and joint territoriality in a simultaneous hermaphrodite: the coral reef fish *Serranus tigrinus. Zeitschrift für Tierpsychologie* 56:33-46.

Pruvot-Fol, A. 1961. Les organes genitaux des opisthobranches. *Archives de Zoologie experimentelle et general.* 99:185-224.

Reeder, R. L. 1986. A new species of *Helminthoglypta* (Gastropoda: Pulmonata: Helminthoglyptidae) from the Cuyamaca mountains of Southern California. *Veliger* 28:444-447.

Reinboth, R. 1962. Morphologische and funktionelle Zweigeschlechtlichkeit bei marinen Telostieren (Serranidae, Sparidae, Centracanthidae, Labridae). *Zoologische Jahrbücher, Physiologie* 69:405-480.

Ricketts, E. F. and J. Calvin, revised by J. W. Hedgpeth. 1968. *Between Pacific Tides.* Stanford University Press, Stanford, California. 614 pp.

Riechert, S. E. and P. Hammerstein. 1983. Game theory in the ecological context. *Annual Review of Ecology and Systematics* 14:377-409.

Rivest, B. R. 1984. Copulation by hypodermic injection in the nudibranchs *Palio zosterae* and *P. dubia* (Gastropoda, Opisthobranchia). *Biological Bulletin* 167:543-554.

Rose, R. A. and I. Hoegh-Guldberg. 1982. A brood-protecting nudibranch with pelagic lecithotrophic development. *Journal of Molluscan Studies* 48:231-232.

Rudman, W. B. 1974. A comparison of *Chelidonura, Navanax* and *Aglaja* with other genera of the Aglajidae (Opisthobranchia: Gastropoda). *Zoological Journal of the Linnean Society* 54:185-212.

Rudman, W. B. 1981. Further studies on the anatomy and ecology of opisthobranch molluscs feeding on the scleractinian coral *Porites. Zoological Journal of the Linnean Society* 71:373-412.

Rudolph, P. H. 1979a. The strategy of copulation of *Stagnicola elodes* (Say) (Basommatophora: Lymnaeidae). *Malacologia* 18:381-389.

Rudolph, P. H. 1979b. An analysis of copulation in *Bulinus (Physopsis) globosus* (Gastropoda: Planorbidae). *Malacologia* 19:147-155.

Rudolph, P. H. and J. B. Bailey. 1985. Copulation as females and use of allosperm in the freshwater snail genus *Bulinus* (Gastropoda: Planorbidae). *Journal of Molluscan Studies* 51:267-295.

Rutowski, R. L. 1983. Mating and egg mass production in the aeolid nudibranch *Hermissenda crassicornis* (Gastropoda: Opisthobranchia). *Biological Bulletin* 165:296-285.

Sella, G. 1985. Reciprocal egg trading and brood care in a hermaphroditic polychaete worm. *Animal Behaviour* 33:938-944.

Sella, G. 1988. Reciprocation, reproductive success and safeguards against cheating in a hermaphroditic polychaete worm, *Ophryotrocha diadema* Åkesson, 1976. *Biological Bulletin* 175:212-217.

Shapiro, D. Y. and R. H. Boulon, Jr. 1982. The influence of females on the initiation of female-to-male sex changes in a coral reef fish. *Hormones and Behavior* 16:66-75.

Sutherland, W. J. 1986. Measures of sexual selection. *Oxford Surveys in Evolutionary Biology* (1985) 2:90-91.

Tompa, A. S. 1980. The ultrastructure and mineralogy of the dart from *Philomycus carolinianus* (Pulmonata: Gastropoda) with a brief survey of the occurrence of darts in land snails. *Veliger* 23:35-42.

Tompa, A. S. 1984. Land Snails (Stylommatophora). *In: The Mollusca,* Vol. 7, *Reproduction.* A. S. Tompa, N. H. Verdonk and J. A. M. van der Biggelaar, eds. pp. 47-140. Academic Press, Orlando, Florida.

Tompa, A. S., N. H. Verdonk and J. A. M. van den Biggelaar (eds.). 1984. *In: The Mollusca,* Vol. 7, *Reproduction.* Academic Press, Orlando, Florida. 486 pp.

Trivers, R. L. 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology* 46:35-37.

Trivers, R. L. 1972. Parental investment and sexual selection. *In: Sexual Selection and the Descent of Man.* B. Campbell, ed. pp. 136-176. Aldine Publishing Company, Chicago.

Warner, R. R. 1982. Mating systems, sex change and sexual demography in the rainbow wrasse, *Thalassoma lucasanum. Copeia* 1982:653-661.

Warner, R. R. and P. Lejeune. 1985. Sex change limited by paternal care: a test using four Mediterranean labrid fishes, genus *Symphodus. Marine Biology* 87:89-99.

Wawra, E. 1988. *Strubellia paradoxa* (Strubell, 1892) (Gastropoda: Opisthobranchia) von den Salomon-Inseln. *Zoologischer Anzeiger* 220:163-172.

Webb, G. R. 1948. The mating of *Stenotrema fraternum* (Say). *Nautilus* 62:8-12.

Wesenberg-Lund, C. 1939. *Biologie der Süsswassertiere, Wirbellose Tiere.* Verlag Julius Springer, Vienna. 817 pp.

Williams, G. C. 1975. *Sex and Evolution.* Princeton University Press, Princeton, New Jersey. 200 pp.

Willson, M. F. and N. Burley. 1983. *Mate Choice in Plants.* Princeton University Press, Princeton, New Jersey. 244 pp.

Date of manuscript acceptance: 16 November 1990

Substratum associations of natural populations of Iceland Scallops, *Chlamys islandica* Müller 1776, on the northeastern Grand Bank of Newfoundland

Kent D. Gilkinson[1] **and Jean- Marc Gagnon**[2]

[1]LeDrew, Fudge and Associates Limited, P. O. Box 9370 Sta. B, St. John's, Newfoundland, Canada A1A 2Y3
[2]Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

Abstract. Live, Iceland scallops, *Chlamys islandica*, were enumerated and their occurrences assigned to substratum coarseness grades along five photographic transects (8-10 km in length) covering areas of the northeastern Grand Bank of Newfoundland. Scallops were disproportionately (53-94%) associated with the coarsest grade of substratum comprising dense gravel-cobble (80-100% by area). Overall, scallops were uncommon to rare on predominantly sand substrata. Average densities of scallops per photograph (5.4 m^2) ranged from 0.5 to 13.8 in cobble fields and from 0.02 to 1.7 on open sand.

It is hypothesized that Iceland scallops on the northeastern Grand Bank are aggregated on coarse substrata because of a strong propensity towards byssal attachment at all post-larval life history stages. A survey of substratum associations of extant species of *Chlamys* reveals that, with few exceptions, association with coarse substrata is common within the genus.

The Iceland scallop *Chlamys islandica* Müller, 1776, is a subarctic-boreal species, extending from Hudson Strait, N.W.T., south to the Massachusetts region (Lubinsky, 1980). Its bathymetric range extends to about 180 m and over the expansive Newfoundland continental shelf, it can occur at commercial densities (Naidu and Cahill, 1989). Apart from mostly anecdotal accounts, very little is known about substratum associations of natural populations of this species and the family Pectinidae in general. To date, research on substrata associations has focused on the settlement and growth of pectinid spat on artificial collectors within an overall aquaculture context. While it is generally known that pectinid spat will settle on substrata such as algae, hydrozoans, bryozoans and various artificial surfaces (Fraser, 1983), the natural settlement substrata for *C. islandica* are unknown (Wallace, 1982). Subsequent to spat settlement, it is believed that juvenile scallops display an overall movement from primary settlement substrata to substrata where they will reside during juvenile and adult stages. In the case of *C. islandica*, this could take place about one year after settlement when they have attained a shell height of >5 mm (Wallace, 1982).

Based on the contents of scallop dredges, Naidu (1988) found that Newfoundland populations of Iceland scallops are found normally at depths greater than 55 m, usually on hard bottom of variable substratum composition including mixtures of sand, gravel, shell fragments, rocks and boulders. Dense, commercial concentrations of Iceland scallops are known to occur on St. Pierre Bank (Newfoundland Grand Banks) (Naidu and Cahill, 1989) in areas with sediments characterized as Sable Is. gravel, which is a mixture of gravel and < 10% sand (Fader *et al.*, 1982). Most of the specimens of North American continental shelf *Chlamys islandica* in the collection of the National Marine Fisheries Service (Woods Hole) were collected from coarse substrata comprising gravel, sand-gravel, till and sand (Theroux and Wigley, 1983). Wiborg (1962, cited in Vahl and Clausen 1980) stated that *C. islandica* lives on coarse sediments or on hard bottom. In a shallow water study in the Gulf of St. Lawrence, Jalbert *et al.* (1989) determined that *C. islandica* occurred most frequently on coarse substrata, primarily cobble and gravel.

The objective of this investigation was to identify natural substratum associations in offshore populations of Iceland scallops from the Newfoundland Grand Bank. Based on a series of photographic transects across areas of the northeastern Grand Bank, spatial distribution patterns of the megafauna and major substratum types (coarseness grades) were described (Schneider *et al.*, 1987). The scope of this paper is threefold. First, Iceland scallop-substrata associations are documented for the northeastern Grand Bank. This is followed by an assessment of physical and behavioural mechanisms which could potentially influence these associations. Finally, observed patterns of substrata association in *Chlamys islandica* are compared with existing information on substrata and habitats occupied by extant *Chlamys* spp.

STUDY AREA

The Grand Banks of Newfoundland represents the most extensive shallow (< 200 m) feature on the continental

American Malacological Bulletin, Vol. 9(1) (1991):59-67

shelf of eastern Canada and is comprised of a series of major banks: St. Pierre, Green, Whale and Grand. The study area is situated on the northeastern edge of the Grand Bank of Newfoundland in the vicinity of the Hibernia oilfield (Fig. 1). Water depths range from 70 to 100 m. The most complete description of this area in terms of the surficial sediments and seabed processes is found in Barrie *et al.* (1984). Sedimentary cover typically consists of reworked sand and gravel deposits, generally < 2 m thick. Bedforms include sand ripples (≤ 1 m wide), sand megaripples (5-10 m wide), sand ribbons (100 m to 1 km wide) and sand ridges (generally > 3 km in width). Coarse sediment is incorporated into the sand, in places. Coarse sediment consists of gravel and cobble-sized clasts (Grand Bank Gravel) which are believed to represent Pleistocene glacial deposits which have been reworked subsequently into coastal environments by an early Holocene marine transgression as a result of a eustatic rise in sea level. Overall, sedimentary bedforms on the Grand Banks are believed to be dynamic and are being reworked by unidirectional storm-driven currents, ocean currents and extreme waves.

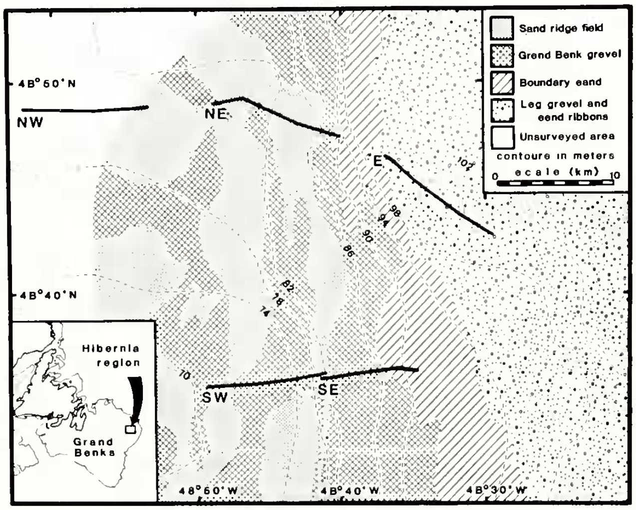

Fig. 1. Location of photographic transects on the northeastern Grand Bank.

METHODS

Five photographic transects, ranging in length from 8 to 10 km, were conducted across the northeastern edge of the Grand Bank (Fig. 1). Colour 35 mm slide photographs were taken at 10 s intervals with the BRUTIV system (Vilks, 1984) which consists of a sled-mounted camera (aligned vertically) towed 3 m above the seabed. Technical details are provided in Schneider *et al.* (1987). The area defined by each photograph was 5.4 m^2.

Each frame (slide) was examined under low magnification (X16) and classified into one of six substratum categories based on a gross classification scheme of sediment texture and estimated areal coverage:
1- pure sand;
2- sand with scattered cobble;
3- scattered gravel on sand;
4- sand with cobble and shell;
5- 50 to 80% cobble and gravel;
6- > 80% cobble and gravel.
Sand and gravel were distinguished on the basis of textural differences. While gravel is defined as particles with diameter > 5 mm (ASTM, 1988), much of the sediment in categories 5 and 6 were dominated by large-sized rocks (> 20 mm diameter) and are referred to as cobble.

All identifiable Iceland scallops were enumerated by superimposing a 5 X 5 grid over each slide and recording the number of organisms in each cell. Analyses were carried out on the sum of counts in each slide. Megafauna less than about 2 cm in shell height were visible, but generally could not be identified to the species level. Scallops were classified as live if they were in normal life position (epifaunal) with normal colouration (i.e. not bleached). While there could be a tendency to overestimate the abundance of live scallops by inclusion of articulated, collapsed cluckers (*sensu* Naidu, 1988), there were probably instances where live, "bleached-looking" scallops were classified as dead. All identifiable scallops displayed a sculpture of coarse ribs and had unequal hinge "ears," features which distinguish the Iceland scallop from the sea scallop *Placopecten magellanicus* (Gmelin, 1791). The only scallop reported from the northeastern Grand Bank has been the Iceland scallop (Mobil, 1985).

For each transect, the frequency distributions of live scallops, by substratum type, were tested statistically for significant departures from random distributions based on the proportion of available substratum types (G-test for goodness of fit, Sokal and Rohlf, 1981). It was assumed that scallops would be distributed randomly at similar densities for all substratum types.

RESULTS

The bedforms which the photographic transects crossed are shown in figure 1. Transect Northwest (NW), which over most of its length traversed previously unsurveyed territory, crossed a sand ridge field with areas of scattered cobble and gravel. Transect Northeast (NE) intersected two sand ridge fields with minor amounts of cobble, gravel and shell. Transect East (E) crossed sand ribbons developed on top of a lag gravel. Transect Southwest (SW) crossed a gravel and cobble field (Grand Bank gravel) with a regular alternation of sand and cobble and Transect Southeast (SE) crossed a gravel and cobble field (Grand Bank gravel) which intersected a sand field (boundary sand) at its eastern end.

Substratum composition varied between transects (Fig.

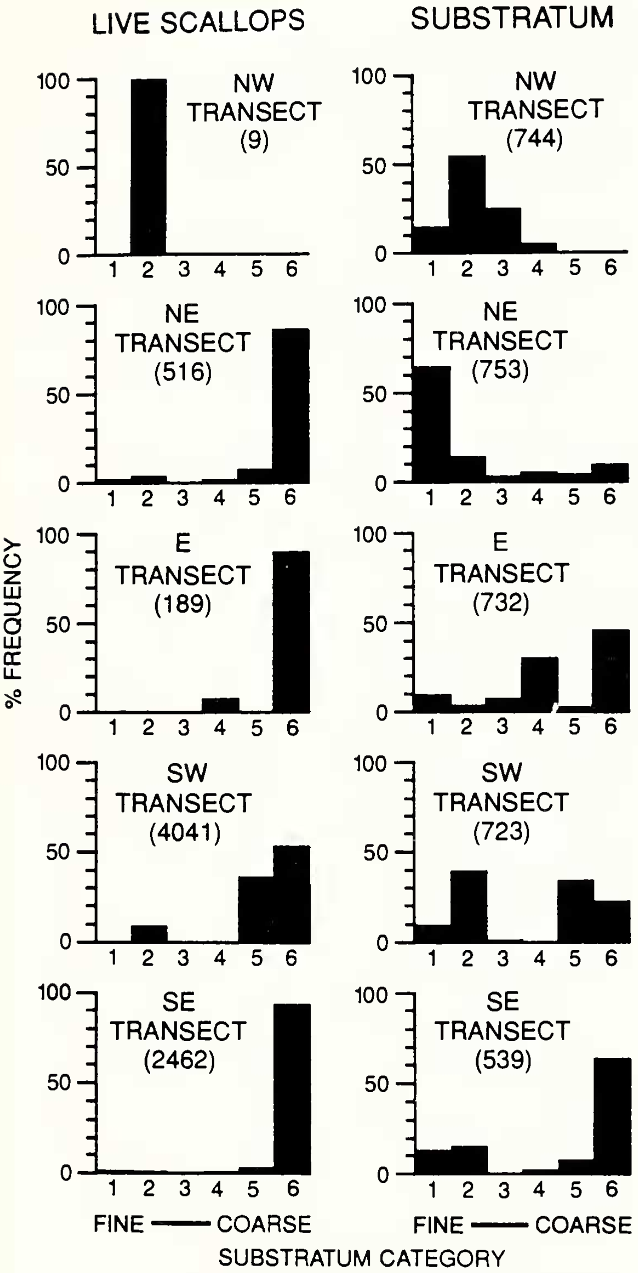

Fig. 2. Frequencies of occurrence of (i) live Iceland scallops by substratum category and (ii) substratum categories along photographic transects. Numbers in parentheses refer to total number of enumerated live scallops (left column) and photographs examined (right column) along each transect.

2). Transects E and SE had a high percentage (50-60%) occurrence of predominantly coarse substrata, comprised of gravel and cobble (some shell), whereas Transects NE and NW had high occurrences of primarily sand substrata (> 60% in both cases). Along Transect SW there was more or less equal representation of fine and coarse substrata.

Along those transects which included the two coarsest substratum categories, scallops were aggregated on coarse substrata (i.e. gravel and cobble) (Fig. 2). Along Transects NE, E and SE, greater than 80% of the total number of live scallops occurred on the coarsest substratrum (category #6). This was in spite of the fact that predominantly fine substrata occurred at frequencies ranging from 15 to 80% along these transects. In particular, along Transect NE where the pure sand substratrum category (#l) had a frequency of occurrence of 65%, approximately 85% of the scallops occurred on cobble substrata which had a frequency of occurence of only 10%. Along Transect SW, approximately 10% of the scallops were counted from substrata comprised predominantly of sand and scattered cobble (Fig. 2). It is noted that this occurred along the transect with the highest densities of live scallops (Fig. 3). Frequencies of scallops along these transects showed a highly significant deviation from a random distribution across all substratum types (G-test statistic range: 176-2869, $p < < 0.01$). Transect NW crossed a sand ridge field with areas of scattered gravel. Along this entire 10 km transect, represented by 744 photographs, only 9 live scallops were counted.

There was considerable variation in the substratum-specific densities of live scallops between transects (Fig. 3). Average densities on the coarsest substratum (> 80% cobble and gravel) ranged from 0.5 to 13.8 scallops per photograph (0.09 to 2.5 scallops/m^2) while average densities on the next coarsest substratum (50% to 80% cobble and gravel) ranged from 0.1 to 5.7 scallops per photograph (0.02 to 1 scallop/m^2). The highest densities on fine substrata occurred on Transect SW with an average density of 1.7 scallops per photograph (0.3 scallops/m^2) on a substratum consisting primarily of open sand with scattered cobble. It was noted previously that this transect displayed the highest densities of scallops on most substratum categories. On the remaining transects, densities ranged from 0.02 to 0.4 scallops per photograph (0.003 to 0.07 scallops/m^2) on the two finest substratum categories.

DISCUSSION

It is obvious that there is a strong association between Iceland scallops and coarse substrata (gravel, cobble) on the northeastern Grand Bank. At the outset, there are several explanations which could account for the observed aggregated distribution of scallops on coarse substrata. These include (1) substratum-specific predation pressure and (2) behavioural and physical mechanisms maintaining scallop-substratum associations.

PREDATORS

The underlying premise of substratum-specific predation pressure is that scallops suffer heavy mortality from predators after movement of juveniles or adults onto fine

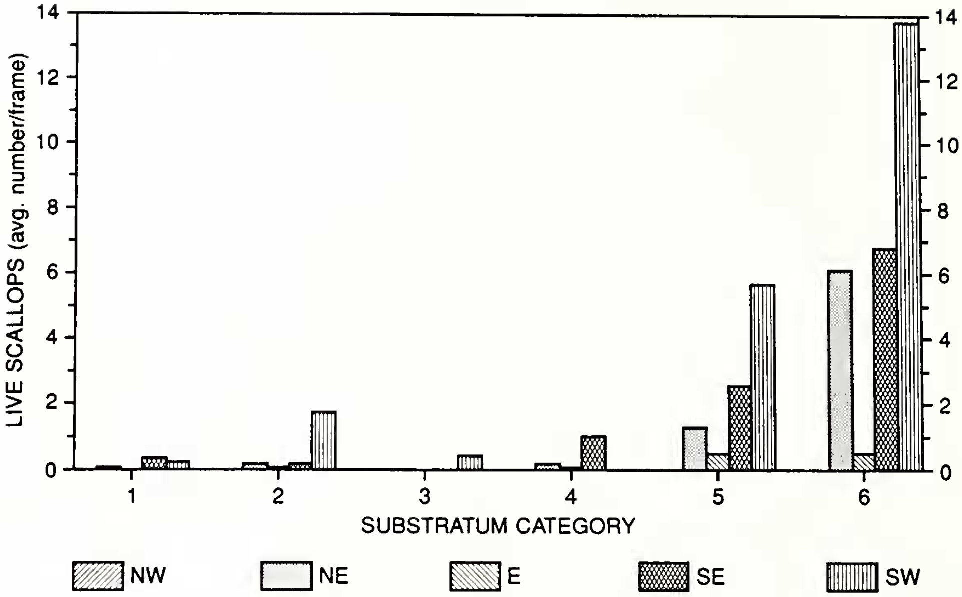

Fig. 3. Average densities of live Iceland scallops by substratum type along photographic transects.

substrata. As mentioned previously, evidence to date on pectinid spat substratum preference rules out primary settlement on fine substrata (i.e. sand). While disproportionate predation pressure due to predator-substrata associations cannot be ruled out as a contributing factor, we believe that predators on the northeastern Grand Bank are not responsible for the skewed distribution of scallops on coarse vs. fine substrata.

Potential predators of Iceland scallops on the Grand Banks are listed in Table 1. Of these, only the pleuronectids, American plaice (*Hippoglossoides platessoides* Fabricius, 1780) and yellowtail flounder (*Limanda ferruginea* Storer, 1839), are known predators of Iceland scallops on the Grand Banks (Pitt, 1976; Naidu and Meron, 1986) and other continental shelf regions (Langton and Bowman, 1981). Naidu and Meron (1986) determined that Iceland scallops occurred in plaice stomachs with a frequency of 22% on St. Pierre Bank. They found that Iceland scallops were susceptible to predation until the age of five years, at which point they achieved a size refuge which was a function of predator mouth gape. While probably not accounting for the rarity of scallops on fine substrata on the northeastern Grand Bank, American plaice would enhance the contrast in distribution of scallops between coarse and fine substrata through predation on these relatively rare occurring individuals on fine substrata.

Other large predatory fish such as Atlantic cod (*Gadus morhua* Linnaeus, 1758) are not known to be associated with any particular substratum type and are typical opportunistic feeders. Examination of cod stomach contents from the Grand Banks reveals a very low incidence of Iceland scallops (G. Lilly, pers. comm.).

Little is known about invertebrate predators of Iceland scallops. The Buccinidae and the Naticidae are probably the two major predatory gastropod groups on the Grand Banks. Species lists are incomplete for the study area and ecological relationships are poorly documented. However, from studies conducted in coastal areas, it is known that adult *Buccinum undatum* (Linnaeus, 1758) can be attracted over considerable distances (> 50 m/day) in search of bivalves which are their primary prey, on substrata including sand, mud and rock

Table 1. Potential predators of the Iceland scallop, *Chlamys islandica*, on the northeastern Grand Bank of Newfoundland.

Predator[1]	Substratum Fine	 Coarse
MOLLUSCA		
Buccinidae	P	P
Naticidae	P*	
CRUSTACEA		
Majidae- includes *Hyas* spp., and *Chionocetes opilio*	P	P
ECHINODERMATA		
Asteroidea		
Asterias vulgaris		P*
Leptasterias sp.		P*
CHORDATA		
Rajidae	P*	
Gadidae	P	P
Zoarcidae	P	P
Cottidae	P	P
Pleuronectidae	K*	
Pseudopleuronectes americanus		
Limanda ferruginea		

[1] -Identified along photographic transects (Schneider *et al.*, 1987)
* -Typical substratum association
P-potential predator
K-known predators (Langton and Bowman, 1981; Naidu and Meron, 1986)

(Himmelman, 1988; Jalbert *et al.*, 1989). Buccinid snails were common over all photographic transects and substratum types, although never abundant. The maximum average density for a transect was 0.4 snails/photograph (0.07 snails/m^2) (Schneider *et al.*, 1987). Although uncommon in photographs, naticid gastropods were observed along the study transects on sand substrata. Most species of Naticidae are relatively stenotypic and prefer sand or muddy substrata (Golikov and Sirenko, 1988). If these gastropods were exerting a heavy mortality on scallops, one would expect to find a surface accumulation of empty scallop shells; this was not observed. Alternatively, naticids could be preying upon small scallops in the size range below the limits of resolution in photographs (i.e. < 2 cm).

Crabs are known predators of various scallop species (Elner and Jamieson, 1979; Lake *et al.*, 1987). While potentially important predators of scallops such as majid crabs (*Hyas* spp. and *Chionocetes opilio* O. Fabricius, 1780) were common over most transects, they were not restricted to a particular substratum (Schneider *et al.*, 1987). The extent of predation by various echinoderms (e.g. Asteroidea) on scallops is unknown, however, it is noted that asteroids were primarily associated with the coarsest substrata on the transects (Schneider *et al.*, 1987) and, therefore, would not be expected to cause excessive mortalities on sand substrata. In conclusion, it is considered unlikely that Iceland scallops are concentrated on coarse substrata due to differential survivorship from intense predation pressure on fine substrata.

BEHAVIOURAL AND PHYSICAL MECHANISMS MAINTAINING SCALLOP-SUBSTRATUM ASSOCIATIONS

The most plausible explanation for the association of Iceland scallops with coarse substrata is the propensity, at all life history stages, towards byssal attachment to a stable substratum. It is known that a high percentage of individuals in a population of Iceland scallops are attached to the substratum by the byssus at any given time. Frequencies of 76% (laboratory) and 97% (field) byssally attached adult scallops have been reported by Naidu and Meron (1986) and Vahl and Clausen (1980), respectively. From diving observations in West Greenland, Pedersen (1989) reported that Iceland scallops were attached to the substratum by the byssus, large scallops were attached directly to the substratum while small scallops were attached to larger scallops or empty shells. Vahl and Clausen (1980) postulate that because *Chlamys islandica* cannot recess on coarse sediments, it remains in danger of being swept away by currents and because *C. islandica* tends to occur in habitats with strong currents (Wiborg, 1962 *fide* Vahl and Clausen, 1980) byssal attachment remains necessary at all sizes.

An important aspect in assessing the importance of water movements in shaping the distribution of scallops is the current speed required to dislodge byssally attached scallops. Gruffydd (1976) determined "wash-away" velocities for Iceland scallops which ranged from 21 cm/s for 10-20 mm individuals to 26 cm/s for 65-70 mm individuals. For the northeastern Grand Bank of Newfoundland, Barrie *et al.* (1984) hypothesized that periodic, high unidirectional flow velocities (> 50 cm/s) occur, possibly every year to every few years at depths less than 110 m. Under these extreme flow conditions, the coarsest sediments become mobile and move as bedforms. At other times, maximum tidal current velocities (15 cm/s, Mobil, 1985) in the study area are less than those required to "wash-away" scallops. Therefore, within the study area, scallops are probably washed away infrequently although major storms would have the capability of dislodging large numbers of scallops. At present, the extent of the impact of such extreme events is unknown.

Swimming activity would make *Chlamys islandica* susceptible to being "washed-away" from preferred substrata. Gruffydd (1976) determined that all sizes of Iceland scallops, and particularly medium-sized (30-40 mm shell height) individuals, displayed a tendency to swim. However, Vahl and Clausen (1980) considered swimming activity to be a relatively rare phenomenon, with individual scallops making, on average, a swimming excursion every 31 days. This is in spite of the fact that byssus production is a minor item in the energy budget of *C. islandica* (Vahl and Clausen, 1980). There are limited data regarding conditions which initiate the swimming response. While Gruffydd's (1976) experiments showed that the swimming response was strongest at the fastest current speed (15 cm/s), Vahl and Clausen (1980) determined the flight reaction to be less evident during periods when current speeds were strong (about 50 cm/s) and speculated that this was probably due to the high risk to scallops associated with being carried to unsuitable habitats in strong currents. Byssal attachment rate of adult *C. tehuelcha* (d'Orbigny, 1835) increased dramatically over relatively small changes in current velocity, from 65% attachment at 6.6 cm/s to 90% at 8.3 cm/s (Ciocco *et al.*, 1983 *fide* Orensanz *et al.*, in press). Patterns of swimming behaviour in *C. islandica* may be determined by a combination of factors including habitat type and critical current velocity (in terms of initiating the swimming response) specific to these habitat types.

SUBSTRATA AND HABITATS OCCUPIED BY EXTANT *CHLAMYS* SPP.

In the evolution of the Bivalvia, neotenous retention of the byssus was an adaptive break-through in terms of physical stabilization, giving rise to an invasion of new habitats by epifaunal species (Stanley, 1972). The oldest (Triassic) pectinids are of the adult-byssate *Chlamys* type (Triassic) while the emergence of post-Triassic free-living pectinids evolved from byssate forms (Stanley, 1972).

Table 2. Approximate maximum sizes (shell height) and substrata and depths occupied by extant *Chlamys* spp.[1]

	Shell Height (mm)	Habitat Substratum	Depth[2]	Source
NORTHWEST ATLANTIC				
Chlamys islandica (Müller, 1776)	100	gravelly sand, shell rock	offshore (to 220 m)	this study; Theroux and Wigley, 1983; Naidu and Cahill, 1989
SOUTHWEST ATLANTIC				
C. benedicti (Verrill and Bush, 1897)	13	?	?	Abbott, 1974
C. mildredae (Bayer, 1943)	38	undersides of rocks	inshore (upper subtidal)	Abbott, 1974
C. sentis (Reeve, 1853)	38	undersides of rocks	inshore (< 15 m)	Abbott, 1974; Rehder, 1981
C. ornata (Lamarck, 1819)	40	undersides of rocks	inshore (to 4 m)	Abbott and Dance, 1986
C. imbricata (Gmelin, 1791)	44	undersides of rocks	inshore (< 6 m)	Abbott, 1974
C. multisquamata (Dunker, 1864)	59	rock crevices	inshore/offshore (6-56 m)	Abbott, 1974
C. patagonica (King and Broderip, 1832)	79	consolidated sand, shell	offshore (to 300 m)	Orensanz *et al.*, in press; O. Iribarne, pers. comm.
C. tehuelcha (d'Orbigny, 1835)	100	consolidated sand; shell-gravel, rocky bottoms	inshore/offshore (< 60 m)	Orensanz *et al.*, in press; O. Iribarne, pers. comm.
EASTERN ATLANTIC				
C. furtiva (Loven)	19	muddy, gravelly sand	inshore/offshore (< 200 m)	Tebble, 1966
C. striata (Müller, 1776)	19	muddy sand, gravel, shell	inshore/offshore	Tebble, 1966
C. tigerina (Müller, 1776)	25	sandy mud, gravel, rock	inshore/offshore (to 550 m)	Madson, 1949; Tebble, 1966
C. multistriata (Poli, 1795)	30	?	inshore/offshore (to 2000 m)	Abbott and Dance, 1986
C. tincta (Reeve, 1853)	30	?	?	Abbott and Dance, 1986
C. sulcata (Müller, 1776)	40	?	offshore (to 850 m)	Abbott and Dance, 1986
C. distorta (da Costa)	50	?	inshore/offshore (to > 90 m)	Tebble, 1966
C. flabellum (Gmelin, 1791)	50	?	inshore	Abbott and Dance, 1986
C. septemradiata (Müller, 1776)	51	mud	inshore/offshore (11-183 m)	Allen, 1953; Tebble, 1966
C. nivea (MacGillivray, 1825)	60	?	offshore	Abbott and Dance, 1986
C. varia (Linnaeus, 1758)	64	rocks, muddy gravel, shell	inshore/offshore (to 1000 m)	Allen, 1953; Tebble, 1966
ICELAND				
C. islandica (Müller, 1776)	110	clay, sand, shell	inshore/offshore (to 300 m)	Madson, 1949
BERING SEA				
C. behringiana (Middendorff, 1849)	?	?	offshore (40-150 m)	Bernard, 1983
C. pseudislandica (MacNeil, 1967)[3]	75	?	inshore/offshore	MacGinitie, 1959; Bernard, 1979
EASTERN PACIFIC				
C. jordani (Arnold, 1903)	?	?	inshore/offshore (2-60 m)	Bernard, 1983
C. lowei (Hertlein, 1935)	?	?	inshore/offshore (2-175 m)	Bernard, 1983; Keen, 1971
C. amandi (Hertlein, 1935)	40	?	offshore	Abbott and Dance, 1986
C. incantata (Hertlein, 1972)	60	?offshore (200 m)	Abbott and Dance, 1986	
C. rubida (Hinds, 1845)	60	rocks, gravel, shell	inshore/offshore (to 183 m)	Rehder, 1981; Kozloff, 1983
C. hastata hastata (Sowerby, 1842)	64	rocks, gravel	inshore/offshore (5-150 m)	Rehder, 1981; Bourne, 1987
C. hastata hericius (Gould, 1850)	83	rocks, sand, mud	inshore/offshore (to 152 m)	Rehder, 1981
SOUTHWEST PACIFIC				
C. dichroa (Suter, 1909)	42	?	offshore (to 100 m)	Abbott and Dance, 1986; Powell, 1979
C. zelandiae (Gray, 1843)	30	undersides of rocks	inshore (to 30 m)	Abbott and Dance, 1986
C. gemmulata (Reeve, 1853)	30	?	inshore (to 30 m)	Abbott and Dance, 1986
C. kiwaensis (Powell, 1933)	33	?	inshore?/offshore	Powell, 1979
C. zeelandona (Hertlein, 1931)	35?	?	inshore	Powell, 1979
C. atkinos (Petterd, 1886)	38	?	inshore/offshore?	MacPherson and Gabriel, 1962
C. luculenta (Reeve, 1853)	40	?	offshore	Abbott and Dance, 1986
C. lentiginosa (Reeve, 1853)	40	coral reefs	inshore	Abbott and Dance, 1986
C. taiaroa (Powell, 1952)	43	?	inshore?/offshore	Powell, 1979
C. funebris (Reeve, 1853)	50	?	inshore?	Abbott and Dance, 1986
C. australis (Sowerby, 1847)	60	?	offshore	Abbott and Dance, 1986

Table 2. (continued)

	Shell Height (mm)	Habitat Substratum	Depth[2]	Source
C. scabricostata (Sowerby, 1915)	60	?	offshore	Abbott and Dance, 1986
C. squamosa (Gmelin, 1791)	60	?	inshore	Abbott and Dance, 1986
C. dieffenbachi (Reeve, 1853)	64	?	inshore/offshore (to 35 m)	Abbott and Dance, 1986; Powell, 1979
C. delicutula (Hutton, 1873)	70	gravel and shell	offshore (to 200 m)	Bull, in press
C. asperrimus (Lamarck, 1819)	100	muddy sand to sand[4]	inshore/offshore (to 100 m)	Young and Martin, 1989; R. McLoughlin, pers. comm.
C. bifrons (Lamarck, 1819)	150	sandy and coarse bottoms	inshore/offshore (to 100 m)	Young and Martin, 1989; R. McLoughlin, pers. comm.
C. consociata (E. A. Smith, 1915)	?	?	inshore/offshore (to 182 m)	Powell, 1979
NORTHWEST PACIFIC				
C. albida (Arnold, 1906)	?	?	offshore (to 200 m)	Bernard, 1983
C. princessae (Kuroda and Habe)	23?	sand, shell	offhsore (to 200 m)	Kuroda *et al.*, 1971
C. asperulata (Adams and Reeve, 1850)	25	?	inshore (to 20 m)	Abbott and Dance, 1986
C. albolineata (Sowerby, 1887)	25	?	inshore	Abbott and Dance, 1986
C. empressae (Kuroda and Habe)	30?	sand, shell	offshore (to 200 m)	Kuroda *et al.*, 1971
C. irregularis (Sowerby, 1842)	40	rocks, gravel	inshore/offshore (to 600 m)	Abbott and Dance, 1986; Kuroda *et al.*, 1971
C. jousseaumei (Bavay, 1904)	40	fine sand	inshore/offshore	Abbott and Dance, 1986; Kuroda *et al.*, 1971
C. larvata (Reeve, 1853)	40	?	offshore	Abbott and Dance, 1986
C. farreri nipponensis (Kuroda)	?	rock, gravel	inshore/offshore (to 60 m)	Kuroda *et al.*, 1971
C. lemniscata (Reeve, 1853)	50	sand, shell	inshore/offshore (to 300 m)	Abbott and Dance, 1986; Kuroda *et al.*, 1971
C. squamata (Gmelin, 1791)	75	rock, gravel	inshore/offshore (to 50 m)	Abbott and Dance, 1986; Kuroda *et al.*, 1971
C. gloriosa (Reeve, 1852)	75	?	offshore	Abbott and Dance, 1986)
C. rosealbus (Scarlato)	90	silty-sand with pebbles, rocks (rarely sand, shells)	inshore/offshore (13-2030 m)	Silina and Pozdnyakova, 1990
C. nobilis (Reeve, 1852)	118?	rocks	inshore (to 20 m)	Kuroda *et al.*, 1971
INDIAN OCEAN				
C. ruschenbergerii (Tyron, 1869)	75	?	offshore	Abbott and Dance, 1986
C. senatoria (Gmelin, 1791)	75	?	offshore	Abbott and Dance, 1986
C. townsendi (Sowerby, 1895)	150	?	inshore (to 20 m)	Abbott and Dance, 1986

[1] This is not a complete taxonomic listing. Bernard (1983) considers *Hinnites* to be a subgenus, however, because *Hinnites* spp. attach to the substrate by cementation rather than by a byssus this group has been excluded from analyses. In certain instances a species may occupy two geographic regions, however, in order to simplify the table the species is recorded for only one region. An exception was made in the case of *C. islandica*.

[2] Those species with maximum depth distributions of 30 m are considered inshore species. Note that this division is arbitrary and that not all deepwater occurrences are necessarily offshore.

[3] There is some debate over whether or not living *C. islandica* occurs in the eastern Pacific although it is reported to occur in the Arctic (Bernard, 1979; Lubinsky, 1980). Bernard (1979) considers *C. islandica* recorded from Point Barrow, Alaska (MacGinitie, 1959) to be *C. pseudislandica* while the more southerly occurring specimens from this collection he considers to be *C. rubida*.

[4] Although widespread on a variety of soft substrata, both juveniles and adults usually found attached by byssus to available solid objects, i.e. rocks, other bivalves, pier pilings (Young and Martin, 1989).

During the Paleozoic, bivalves were primarily restricted to nearshore habitats and it was largely after the Paleozoic that the bivalvia spread offshore to attain their present distributions, replacing the previously dominant articulate brachiopods (Stanley, 1972). From a survey of the habitats occupied by extant species within the genus *Chlamys*, it is seen that there is a high proportion (73%) of species with an offshore distribution, or at least ranging from shallow water to offshore depths (Table 2).

While there is limited ecological information on many of these species, particularly with respect to substrata associations, it would appear that members of the genus have radiated into a variety of habitat types. Substrata associations range from the undersides of shallow water boulders and coral reefs (e.g. subtropical and tropical species) to deepwater muds (e.g. *Chlamys septemradiata* Müller, 1776). Kauffman (1969) classifies byssate species of *Chlamys* as byssate fissure-dwellers. The typical habitats of this group are the undersides of rocks, crevices and fissures, reef tunnels, spaces inside root bundles of aquatic plants and similar niches with good water circulation, weak light and good protection from strong wave or current action. The occurrence of *C. septemradiata* on deep-water, flocculent muds (Allen, 1953) represents a unique substratum association within the genus. In fact, in this habitat, *C. septemradiata* serves as a stable settling surface for other sessile invertebrates which otherwise would be subjected to siltation.

Examining species-specific maximum sizes within the genus, it is seen that while most shallow water crevice species are small (< 60 mm), there are several very small (≤ 30 mm) species which are found distributed offshore to great depths (Table 2). The Iceland scallop is one of the largest species within the genus *Chlamys*, attaining a maximum size of about 100 mm in the Newfoundland regions although on the northeastern Grand Bank most scallops are between 60 and 80 mm in shell height (Naidu and Cahill, 1989).

The observed life habit orientation of *Chlamys islandica* on the northeastern Grand Banks is a fully exposed position. Because of the nature of the substratum (dense gravel, cobble), in most instances adult Iceland scallops must assume an epifaunal position on the exposed, upper surfaces of rocks. Only in the case of irregular occurrences of boulders would Iceland scallops be afforded the opportunity to assume a cryptic habit by attaching to the undersides. However, *C. islandica* could be compensated for this apparent lack of refuge through heavy biofouling by barnacles and soft corals in particular, which is often observed on the external surfaces of the upper valve (KDG, pers. obs.). While this may not decrease the risk of predation from chemosensory orienting predators (see Lake *et al.*, 1987), presumably it would be an advantage in the case of visually cueing predators, in particular, fish. Epizoic associations have been studied for several species. Epizoic sponge cover of the valves of *C. varia* (Linnaeus, 1758) and *C. asperrima* (Lamarck, 1819) is known to provide protection from predatory starfish (Forester, 1979; Chernoff, 1987; Pitcher and Butler, 1987). *Chlamys dieffenbachi* (Reeve, 1853) is almost invariably enveloped in living sponge (Powell, 1979) while *C. hastata* and *C. rubida* (Hinds, 1845) are regularly colonized by sponges that form thick coatings (Kozloff, 1983).

In summary, from the results of this study, and the observations of others, it would appear that *Chlamys islandica* is restricted to habitats with coarse substrata although this includes a range of sediment types from gravelly sand to gravel, cobble and shell mixtures. This would appear to be due to a requirement for byssus attachment at all life history stages. Within the genus, other species known to be bysally attached to substrates as adults include: *C. asperrima* (Young and Martin, 1989), *C. varia* (Rodhouse and Burnell, 1979), *C. irregularis* Sowerby, 1842, *C. squamata* Gmelin, 1791, *C. farreri* Jones and Preston, 1904 and *C. nobilis* Reeve, 1853 (Kuroda *et al.*, 1971). Frequency of byssal attachment to the substratum decreases with age in *C. tehuelcha* although the capacity to form a byssus is not lost in the largest individuals (Orensanz *et al.*, in press). While occupation of habitats with coarse substrata appears to be typical of members of the genus, there is at least one example *(C. septemradiata)* of radiation into a deepwater habitat characterized by flocculent muds, presumably with a consequential loss of byssus attachment.

ACKNOWLEDGMENTS

We thank the Centre for Cold Ocean Research and Engineering (Memorial University of Newfoundland), the Atlantic Geosciences Centre (Bedford Institute of Oceanography, Dartmouth, Nova Scotia), the Offshore Geotechnics Program of the Federal Panel on Energy Research and Development, T. Folkes, M. Lewis and the Captain and crew of the C.S.S. HUDSON for logistic support. We thank J. A. Hutchings and S. Naidu for reviewing the manuscript, G. Carmichael for graphics, and D. Pitcher for assistance with data analyses. We are grateful to S. Shumway, M. Bricelj, O. Iribarne, P. Young and R. McLoughlin for providing unpublished data or information sources for Table 2.

LITERATURE CITED

Abbott, R. T. 1974. *American Seashells. The Marine Mollusca of the Atlantic and Pacific Coasts of North America.* Second Edition. Van Nostrand Reinhold Company, New York. 663 pp.

Abbott, R. T. and P. Dance. 1986. *Compendium of Seashells.* American Malacologists Inc. Melbourne, Florida. 411 pp.

Allen, J. A. 1953. Observations on the epifauna of the deep-water muds of the Clyde Sea area, with special reference to *Chlamys septemradiata* (Müller). *Journal of Animal Ecology* 22(2):240-260.

ASTM. 1988. Standard test methods for classification of soils for engineering purposes. *In:* Annual book of ASTM (American Society for Testing and Materials) standards. Section 4. pp. 283-292. Philadelphia, Pennsylvania.

Barrie, J. V., C. F. M. Lewis, G. B. Fader and L. H. King. 1984. Seabed processes on the northeastern Grand Banks of Newfoundland; modern reworking of relict sediments. *Marine Geology* 57:209-227.

Bernard, F. R. 1979. Bivalve mollusks of the Western Beaufort Sea. *Contributions in Science*, No. 313. Natural History Museum of Los Angeles County. 80 pp.

Bernard, F. R. 1983. Catalogue of the living bivalvia of the eastern Pacific Ocean: Bering Strait to Cape Horn. *Canadian Special Publications of Fisheries and Aquatic Sciences* No. 61, 102 pp.

Bourne, N. 1987. Status of invertebrate fisheries off the Pacific coast of Canada. *Canadian Technical Report of Fisheries and Aquatic Sciences* No. 1576. 158 pp.

Bull, M. In press. New Zealand. *In: Scallops: Biology, Ecology and Aquaculture.* S. E. Shumway, ed. Elsevier Science Publishers, Amsterdam.

Cernohorsky, W. O. 1972. *Marine Shells of the Pacific.* Vol. II. Pacific Publications, Sydney. 411 pp.

Chernoff, H. 1987. Factors affecting mortality of the scallop *Chlamys asperrimus* (Lamarck) and its epizoic sponges in South Australian waters. *Journal of Experimental Marine Biology and Ecology* 109:155-171.

Elner, R. W. and G. S. Jamieson. 1979. Predation of sea scallops, *Placopecten magellanicus*, by the rock crab, *Cancer irroratus*, and the American lobster, *Homarus americanus. Journal of the Fisheries Research Board of Canada* 36:537-543.

Fader, G. B., L. H. King and H. W. Josenhans. 1982. Surficial geology of the Laurentian Channel and the western Grand Banks of Newfoundland. Marine Sciences Paper 21. Geological Survey of Canada Paper 81-22.

Forester, A. J. 1979. The association between the sponge *Halichondria panacea* (Pallis) and scallop *Chlamys varia* (L.): a commensal-protective mutualism. *Journal of Experimental Marine Biology and Ecology* 36:1-10.

Fraser, D. I. 1983. Observations on the settlement of pectinid spat off the west coast of Scotland in 1982. International Council for the Exploration of the Sea. CM 1983/K:40.

Golikov, A. N. and B. I. Sirenko. 1988. The naticid gastropods in the boreal waters of the western Pacific and Arctic Oceans. *Malacological Review* 21:1-41.

Gruffydd, L. D. 1976. Swimming in *Chlamys islandica* in relation to current speed and an investigation of hydrodynamic lift in this and other scallops. *Norwegian Journal of Zoology* 24:365-378.

Himmelman, J. H. 1988. Movement of whelks *(Buccinum undatum)* towards a baited trap. *Marine Biology* 97:521-531.

Jalbert, P., J. H. Himmelman, P. Beland and B. Thomas. 1989. Whelks *(Buccinum undatum)* and other subtidal invertebrate predators in the northern Gulf of St. Lawrence. *Naturaliste Canadian (Revue Ecologie et Systematics)* 116:1-15.

Kauffman, E. G. 1969. Form, Function, and Evolution. *In: Treatise on Invertebrate Paleontology.* R. C. Moore, ed. pp. N130-N205. The Geological Society of America and the University of Kansas.

Keen, A. M. 1971. *Seashells of Tropical West America. Marine Molluscs from Baja California to Peru.* Second Edition, Stanford University Press, Stanford, California. 1064 pp.

Kozloff, E. N. 1983. *Seashore Life of the Northern Pacific Coast. An Illustrated Guide to Northern California, Oregon, Washington and British Columbia.* Douglas and McIntyre, Vancouver/Toronto. 370 pp.

Kuroda, T., T. Habe and K. Oyama. 1971. *The seashells of Sagami Bay. Collected by His Majesty The Emperor of Japan.* Maruzen Co., Ltd., Japan. 489 pp.

Lake, N. C. H., M. B. Jones and J. D. Paul. 1987. Crab predation on scallop *(Pecten maximus)* and its implication for scallop cultivation. *Journal of the Marine Biological Association of the United Kingdom* 67:55-64.

Langton, R. W. and R. E. Bowman. 1981. Food of eight Northwest Atlantic pleuronectiform fishes. National Oceanic and Atmospheric Administration (NOAA) Technical Report NMFS SSRF-749. 16 pp.

Lubinsky, I. 1980. Marine bivalve molluscs of the Canadian central and eastern arctic: faunal composition and zoogeography. *Canadian Bulletin of Fisheries and Aquatic Sciences* No. 207. 111 pp.

MacGinitie, N. 1959. Marine mollusca of Point Barrow, Alaska. *Proceedings of the United States National Museum* 109(3412):59-208, pls. 1-27.

MacPherson, J. H. and C. J. Gabriel. 1962. *Marine Molluscs of Victoria.* Melbourne University Press, Melbourne, Australia. 475 pp.

Madsen, F. J. 1949. *The Zoology of Iceland: Marine Bivalvia.* Volume IV, Part 63. Ejner Munksgaard, Copenhagen and Reykjavik. 85 pp.

Mobil. 1985. Hibernia development project environmental impact statement. Vol. IIIa. Biophysical Environment. Mobil Oil Canada Limited, St. John's, Newfoundland.

Naidu, K. S. 1988. Estimating mortality in the Iceland scallop, *Chlamys islandica* (O. F. Müller). *Journal of Shellfish Research* 7(1):61-71.

Naidu, K. S. and F. M. Cahill. 1989. The quest for Iceland scallops, *Chlamys islandica* (O. F. Müller) on the Grand Bank, NAFO Division 3LNO. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 89/12. 31 pp.

Naidu, K. S. and S. Meron. 1986. Predation of scallops by American plaice and yellowtail flounder. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 86/62. 25 pp.

Orensanz, J. M., M. Pascual and M. Fernandez. In press. Scallop resources from the southwestern Atlantic. *In: Scallops, Biology, Ecology and Aquaculture.* S. E. Shumway, ed., Elsevier Science Publishers, Amsterdam.

Pedersen, S. A. 1989. Inshore scallop resources, *Chlamys islandica*, in the Nuuk Area west Greenland. Northwest Atlantic Scientific Council Research Document 89/20. 16 pp.

Pitcher, C. R. and A. J. Butler. 1987. Predation by asteroids, escape response, and morphometrics of scallops with epizoic sponges. *Journal of Experimental Marine Biology and Ecology* 112:233-249.

Pitt, T. K. 1976. Food of yellowtail flounder on the Grand Bank and a comparison with American Plaice. *International Commission for the the Northwest Atlantic Fisheries Research Bulletin* 12:23-27.

Powell, A. W. B. 1979. *New Zealand Mollusca. Marine, Land and Freshwater Shells.* William Collins Publishers Ltd., Hong Kong. 500 pp.

Rehder, H. A. 1981. *The Audubon Society Field Guide to North American Seashells.* New York. 894 pp.

Rodhouse, P. G. and G. M. Burnell. 1979. *In situ* studies on the scallop *Chlamys varia.* Pp. 87-98. *In:* Progress in Underwater Science Vol. 4. J. C. Gamble and J. D. George, eds. pp. 87-98. Pentech Press, Plymouth, United Kingdom.

Schneider, D. C., J.- M. Gagnon and K. D. Gilkinson. 1987. Patchiness of epibenthic megafauna on the outer Grand Banks of Newfoundland. *Marine Ecology Progress Series* 39:1-13.

Silina, A. V. and L. A. Pozdnyakova. 1990. Growth of the scallop *Chlamys rosealbus* in the Sea of Japan. *The Soviet Journal of Marine Biology* (Translated) 16(1):32-36.

Sokal, R. R. and F. J. Rholf. 1981. *Biometry. The Principles and Practise of Statistics in Biological Research.* Second Edition. W. H. Freeman and Company, San Francisco. 859 pp.

Stanley, S. M. 1972. Functional morphology and evolution of byssally attached bivalve mollusks. *Journal of Paleontology* 46(2):165-212.

Tebble, N. 1966. *British Bivalve Seashells. A Handbook for Identification.* Alden Press, Oxford. 212 pp.

Theroux, R. B. and R. L. Wigley. 1983. Distribution and abundance of east coast bivalve molluscs based on specimens in the National Marine Fisheries Service Woods Hole Collection. NOAA Technical Report NMFS SSRF-768. 172 pp.

Vahl, O. and B. Clausen. 1980. Frequency of swimming and energy coast of byssus production in *Chlamys islandica* (O. F. Müller). *Journal du Conservation International Exploration de la Mer* 39(1):101-103.

Vilks, G. 1984. Report of Cruise 83033, Bedford Institute of Oceanography, Atlantic Geosciences Centre. Geological Survey of Canada internal report, Dartmouth, Nova Scotia. 51 pp.

Wallace, J. C. 1982. The culture of the Iceland scallop, *Chlamys islandica* (O. F. Müller). I. Spat collection and growth during the first year. *Aquaculture* 26:311-320.

Young, P. C. and R. B. Martin. 1989. The scallop fisheries of Australia and their management. *CRC Critical Reviews in Aquatic Sciences* 1(4):615-638.

Date of manuscript acceptance: 1 October 1990

Anatomical and behavioural studies on vision in *Nautilus* and *Octopus*

W. R. A. Muntz
Department of Ecology and Evolutionary Biology, Monash University, Clayton, Victoria 3168, Australia

Abstract. *Nautilus* is a cephalopod that is primitive in many respects, and is often considered to be a "living fossil". The eye of *Nautilus* is apparently a primitive feature, acting as a pin-hole camera and lacking any lens or other dioptric apparatus. In contrast, in *Octopus* and most other coleoid cephalopods, there is a well formed spherical lens. The basic structure of the retina is similar in the two animals, but there are also a number of important differences: the microvilli of the receptors of *Nautilus* do not form a regular rectilinear array as they do in *Octopus*; the microvilli from neighbouring receptors overlap, which does not occur in *Octopus*; the supporting cells have a different structure; the nuclei of the supporting cells and receptor cells are distributed either side of the basement membrane in *Octopus*, but not in *Nautilus*; cilia are present in the retina of *Nautilus* but not *Octopus*; and the myeloid bodies are much more developed in *Nautilus*.

Both behavioural experiments and calculation show that, as expected on anatomical grounds, visual acuity and sensitivity are much better in *Octopus* than *Nautilus*. Reasons for the limitations in the visual capabilities of the two animals are discussed.

Nautilus is the last surviving genus of a group that arose in the Triassic, and has apparently changed little since Cretaceous times, at least as far as we can judge from the shell. The animal shows many apparently primitive features, such as the lack of an ink sac or chromatophores, the external shell, the funnel formed of two overlapping lobes, and the simple pin-hole camera eye lacking any lens or other dioptric apparatus (e.g. Morton, 1967). The ancestry of the genus has been discussed by, among others, Teichert and Matsumoto (1987), who concluded that it can truly be called a "living fossil".

The first octopods, on the other hand, are found in the Upper Cretaceous (Donovan, 1977), and the group must be counted among the most developed invertebrates in existence. The octopus eye, which resembles that of most other coleoid cephalopods, has in contrast to *Nautilus* a very well-developed lens and superficially looks remarkably similar to the eyes of vertebrates. The present paper compares the structure and function of the eyes of these two very different cephalopods.

STRUCTURE OF THE EYE IN *NAUTILUS* AND *OCTOPUS*

The most obvious difference between the eyes of *Nautilus* and *Octopus* is the complete lack of any dioptric apparatus in the former genus. The pupil in *Nautilus* opens directly to the sea, and the eye must act as a pin-hole camera. The eyes of octopuses in contrast have well developed spherical lenses, which, as in most fishes, have focal lengths about 2.5 times their radius (Matthiessen's ratio) and are well corrected for spherical aberration (Sivak, 1982; Sroczyński and Muntz, 1985). The overall size of the eyes of the two animals is, however, similar, and both animals have contractile pupils that are elongated in the horizontal direction (Muntz, 1977; Hurley *et al.*, 1978). Figure 1 shows the general appearance of the eyes of *Nautilus pompilius* Linnaeus and *Octopus vulgaris* Lamarck.

Descriptions of the retinal anatomy of *Nautilus* can be found in Barber and Wright (1969), Muntz and Raj (1984), and Muntz and Wentworth (1987); and of *Octopus* in Young (1962a, 1971) and Yamamoto *et al.* (1965). These papers also give references to earlier work. Following convention, in this paper the segments of the receptors facing the light, which contain the photopigment, will be referred to as the distal or outer segments, and the nuclear region as the proximal or inner segment.

The basic elements of the retina in both *Octopus* and *Nautilus* are the receptor cells, with distal segments consisting of a central core from which the microvilli (which contain the visual pigment) radiate outwards, and the supporting cells with their processes lying between the receptor cell outer segments (Figs. 2, 3). The packing of the receptor cells is roughly similar in the two species. Thus, there are about 20,000 receptor cells mm^{-2} in *N. pompilius*, varying little over the retina (Muntz and Raj, 1984), and between 18,000 and 55,000 mm^{-2} in *O. vulgaris*, depending on retinal position (Young, 1971). Although basically similar, there are however also a number of important differences between the two species, which can be summarised as follows.

American Malacological Bulletin, Vol. 9(1) (1991):69-74

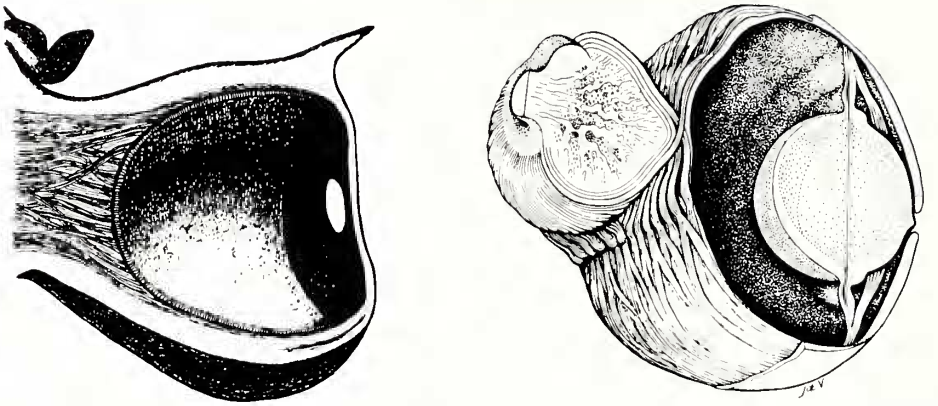

Fig. 1. Left, vertical section through the eye of *Nautilus* (from Willey, 1902). Right, vertical section through the eye and optic lobe of *Octopus* (from Young, 1962b).

(i) Transverse sections through the outer segments of the retinal receptors of *Nautilus* show that there are usually five or six (occasionally four or seven) bundles of microvilli running out from each receptor body to the bodies of neighbouring receptors. The receptors thus form a roughly hexagonal array (Fig. 2a). The microvilli from neighbouring receptors within a given bundle often interdigitate, although the extent of this interdigitation is not clear. The processes of the supporting cells run out in groups between these bundles of microvilli.

In contrast in *Octopus* the receptor outer segments form a rectilinear array, with the microvilli oriented vertically or horizontally with respect to gravity (Fig. 3a). The microvilli of each receptor remain strictly segregated from those of the neighbouring receptors, with no interdigitation.

(ii) The structure of the supporting cells is quite different in the two animals. In *Nautilus* each cell has a number of fine microvillous processes which project out between the receptor outer segments in groups, whereas in *Octopus* each supporting cell has a single process, which is much larger and contains screening pigment. In the former animal the cell nuclei of both the receptors and the supporting cells lie distal to the basement membrane, whereas in *Octopus* the supporting cell nuclei lie distal and the receptor cell nuclei proximal to the basement membrane.

(iii) In *Nautilus* the supporting cells have cilia, as well as the microvillous processes that extend between the receptors. It is not certain whether the receptor cells have cilia as well. Ciliary structures have not been reported in the retina of any other adult cephalopod, although the photosensitive organs of many animals have receptors of ciliary origin, or cilia, presumed not to be photosensitive, intermingled with the receptors (Vanfleteren, 1982).

(iv) The inner segments of *Nautilus* photoreceptors have complex myeloid bodies, which often have the appearance of a tubular structure, or a series of wavy plates (Fig. 2). It has been argued that this apparently complex structure consists of a series of dimpled plates, stacked in register above each other (Muntz and Wentworth, 1987). In *Octopus* and the other coleoid cephalopods, the myeloid bodies are reduced to a few membranous strands.

It is interesting that some of the characteristics by which the *Nautilus* retina differs from that of adult octopuses also have been found during the development of the embryos of coleoid cephalopods. Thus in the cuttlefish *Sepiella japonica* Sasaki, embryos have cilia on both receptor and supporting cells, the nuclei of both the receptor cells and the supporting cells lie distal to the basement membrane, and the supporting cells send long microvillous processes out among the whole length of the receptor outer segments (Yamamoto, 1985). Work in progress shows a similar situation in the embryos of the Australian octopuses *Octopus pallidus* Hoyle and *O. australis* Hoyle (Wentworth and Muntz, unpub. data).

BEHAVIOURAL STUDIES

To date, no studies of vision have been carried out with *Nautilus* using any form of learnt behaviour, and it is not known how far the animals are capable of learning. However, *Nautilus* shows two well developed forms of innate visual behaviour, the positive phototactic response and the optomotor response, which have been used to determine the animals' visual acuity, and also their absolute and spectral sensitivities (Muntz and Raj, 1984; Muntz, 1986, 1987).

As we should expect for an animal with an eye having the simple optics of a pin-hole camera, visual performance in *Nautilus* is very poor compared to that of animals with lens bearing camera eyes. The minimum separable visual acuity, for example, measured using the optomotor response, lies between 5.5^0 and 11.25^0, which agrees well with values calculated on the basis of the gross dimensions of the eye and pupil, and with expectations based on photographing a visual test chart using a scale model of the eye (Muntz and Raj, 1984). This can be compared with values of about 5'

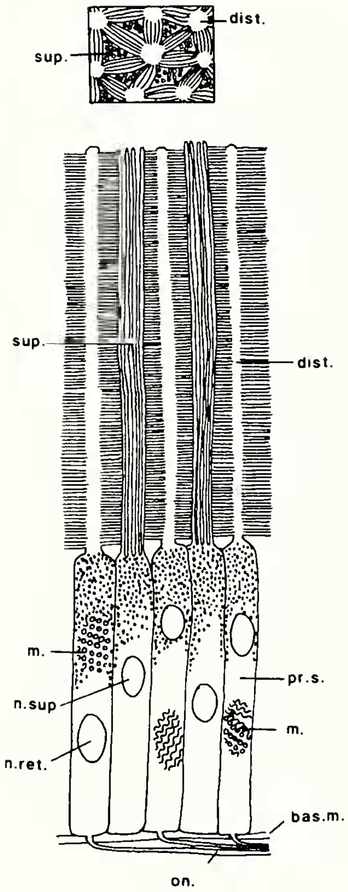

Fig. 2. Diagram of the structure of the retina of *Nautilus*, as seen in tangential (above) and radial section (below). The diagram is not to scale: in particular the horizontal dimensions have been exaggerated compared to the vertical dimensions for clarity. The mean length of the distal segments is in fact about 360 μm, and of the proximal segments 100 μm, and the mean centre to centre distance between adjacent receptors 3.5 μm: there is little variation over the retina (bas.m., basement membrane; dist., receptor distal segment with microvilli radiating from a central core; pr.s., receptor proximal segment; sup., processes of supporting cells; m., myeloid body; on., optic nerves; n.sup., nucleus of supporting cells; n.ret., nucleus of retinal cell).

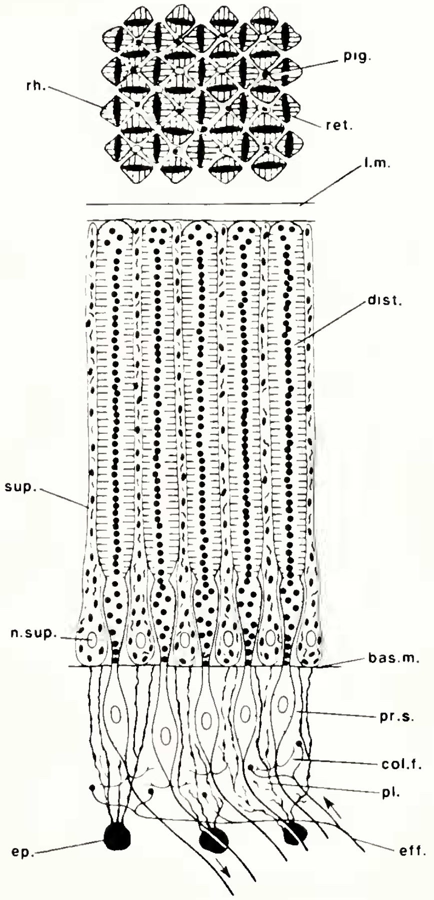

Fig. 3. Diagram of the structure of the retina *Octopus*, as seen in tangential (above) and radial section (below), from Young (1962a). Not to scale: in fact the distal segments vary between about 60 μm and 180 μm in length depending on retinal position, while the proximal segments are about 90 μm long and the individual rhabdomeres about 5 μm in width (Young, 1962a) (rh., rhabdome; pig., pigment granule at centre of rhabdome; ret., retinal cell; l.m., limiting membrane; dist., distal segment of receptor; bas.m., basal membrane; pr.s., proximal segments of retinal cells; col.f., fine dendritic collateral of retina cell; p.l., retinal nerve plexus; eff., ending of efferent fibre in retina; ep., epithelial cell; n.sup., nuclei of supporting cells; sup., processes of supporting cells).

obtained with octopuses, various fishes and two aquatic mammals (Table 1): even with the most favourable estimate of 5.5^0 the performance level of *Nautilus* is over 60 times worse than for these other aquatic animals.

By using the positive phototactic behaviour of *Nautilus* it has also been possible to determine its absolute sensitivity to tungsten light (Muntz, 1987). In itself this result is not particularly useful, because the spectral output of the tungsten source used was very different from that of the light to which the animal will be exposed in its natural environment. However, given a knowledge of the spectral transmission of the water in which the animals live, the spectral quality of the daylight reaching the surface of the sea, and the animals' own spectral sensitivity, it is possible from these results to calculate the maximum depth at which surface light would be visible at all to *Nautilus*. Reasonable estimates for the first two factors are available, and the animals' spectral sensitivity was taken to be the same as the absorption spectrum of its visual pigment (see Muntz, 1987 for details). It appears that some daylight should be visible to *Nautilus* down to 800 m, which is slightly deeper than the maximum depth at which the animal is found. This is, however, considerably less than the maximum depth at which daylight should be visible to deep sea fishes, which has been calculated by Clarke and Denton (1962) as over 1000 m. Calculations based on the dimensions of the *Nautilus* eye also indicate that *Nautilus* will be less sensitive, by about 2 log units, than a fish or cephalopod that has a camera eye with a lens obeying

Matthiessen's ratio (Muntz and Raj, 1984).

Finally, the positive phototactic behaviour has also been used to determine spectral sensitivity directly (Muntz, 1986). The sensitivity curve obtained agreed well with the absorption spectrum of the extractable visual pigment, which was itself well fitted by Dartnall's (1953) visual pigment nomogram for an A_1-based pigment with its maximum at 467 mm.

In contrast to *Nautilus*, octopuses learn visual discriminations very readily, and a great deal of information is now available on their visual capabilities (Wells, 1978; Messenger, 1981 for reviews). Most of this work has concerned higher visual functions, such as the ability to discriminate shapes, the mechanisms by which such discriminations are learnt, and the function of the various parts of the central nervous system. Comparatively little work has been done on the animals' more basic visual capabilities, such as sensitivity or visual acuity, which are probably more directly related to the optics of the eye and the structure and function of the retina, and which can be compared to the data that are available for *Nautilus*. In the case of sensitivity, for example, there appear to have been no studies at all carried out on coleoid cephalopods using learning, and only one study involving innate behaviour, in which the spectral sensitivity of *Loligo pealei* Lesueur larvae was measured using the positive phototactic response in a manner rather similar to that used with *Nautilus*. The results were also similar showing a smooth bell-shaped spectral sensitivity curve maximal at around 480 mm and compatible with a single visual pigment (White, 1924).

A few behavioural studies have been carried out on the visual acuity of octopuses. Thus Sutherland (1963), using a training situation, obtained an estimate of 17' for *Octopus vulgaris*, and Packard (1969), using the same species and the optomotor response, found that in very small specimens (<3-22g) acuity improved with size. The most recent studies on acuity in octopuses (Muntz and Gwyther, 1988a, 1989) used fully grown animals and a two choice learning situation, and the stimuli were gratings of equally spaced black and white stripes oriented vertically, horizontally, or obliquely at 45°. The animals were trained to discriminate these gratings from each other or from a uniform gray stimulus, and visual acuity was taken as the separation between the bars of the gratings where performance reached chance levels. The results showed that the minimum separable visual acuity of *O. australis* and *O. pallidus* is about 5' (Fig. 4). With gratings close to the animals' threshold, performance with the vertical gratings was best, and with the horizontal gratings worst, but the effect was not large.

The ability of *Octopus pallidus* and *O. australis* to discriminate distances has also been determined behaviourally, using the animals' tendency to attack the nearer of two stimuli presented simultaneously (Muntz and Gwyther, 1988b). Assuming that the animals are using accommoda-

Table 1. Minimum separable visual acuities, in minutes of arc, of various aquatic animals measured behaviourally using gratings. Learnt discriminations were used in all cases except *Nautilus* where the optomotor response was used.

Species	Acuity	Reference
MAMMALS		
Harbour seal *Phoca vitulina* Linnaeus	8.3	Schusterman and Balliet, 1970
Stellar Sea Lion *Eumetopias jubata* (Schreber)	7.1	Schusterman and Balliet, 1970
TELEOST FISHES		
Convict fish *Microcanthus strigatus* (Cuvier and Valenciennes)	4.9	Yamanouchi, 1956
Minnow *Phoxinus laevis* Linnaeus	10.8	Brunner, 1934
Skipjack tuna *Katsowomis pelamis* Linnaeus	5.5	Nakamura, 1968
Little tuna *Euthynnus affinis* (Cantor)	7.4	Nakamura, 1968
Cichlid fish *Aequidens portalegrensis* (Hensel)	5.8	Baerends *et al.*, 1960
CEPHALOPODS		
Nautilus *Nautilus pompilius*	330-670	Muntz and Raj, 1984
Octopus *Octopus pallidus* *O. australis*	5.0	Muntz and Gwyther, 1988a

tion to estimate distance, which various tests indicated is the most likely mechanism, the animals can detect blurring of points on the retinal image comparable in size to a single retinal receptor, and lens displacements of around 10 μm.

Finally, training experiments have shown that *Octopus vulgaris* can discriminate the plane of polarised light (Moody and Parriss, 1961), and also it has been shown that two species of decapod larvae orient themselves to the plane of polarised light (Jander *et al.*, 1968). It is not known whether *Nautilus* has this ability.

DISCUSSION

The evolution of the camera eye has attracted interest ever since Darwin [1859 (reprinted 1958)] listed it as one of the "organs of extreme perfection and complication", and wrote that to believe that such organs could have been formed by natural selection seems "absurd in the highest possible degree". Darwin's solution to the problem was to suggest that the eye must have arisen through numerous inheritable gradations, each of which was useful to its possessor, and that while strictly we should look for such gradations among the animal's lineal ancestors, we are usually forced to look at living species of the same group to see what gradations are possible.

The eye of *Nautilus* could be taken as such a gradation on the route to the complex eyes of the more recent cephalopods. Even though *Nautilus* vision is poor, nevertheless its visual behavior is precise, in that in the optomotor response they follow the stripes accurately without visible lag, and in the phototactic situation if the difference between the stimuli is well above threshold the brighter light is chosen on every occasion. The eyes are also stabilised with respect to gravity by means of the statocysts (Hartline *et al.*, 1979). These facts suggest that vision is important to the animal. It is not, however, clear what use they make of such poor vision in their normal life. The habitat of *Nautilus* often has strong currents, and the optomotor behaviour could be related to holding station under these conditions. It could also be that the positive phototactic behaviour is related to bioluminescence, which is a major source of light at depth in the sea. *Nautilus* is often trapped in association with deep water bioluminescent shrimps, which also feed on decaying animal material, and moving towards bioluminescence could help take the animals towards their food. Finally, *Nautilus* shows diurnal vertical migrations (Carlson *et al.*, 1984; Ward *et al.*, 1984), and vision could be a factor in this behaviour. Without further information on the normal behaviour of the animals however, these remain speculations.

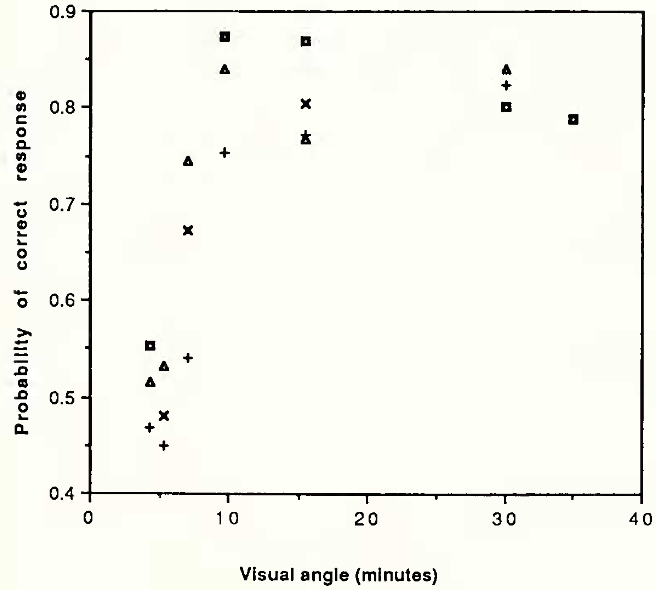

Fig. 4. Visual acuity of *Octopus*, measured behaviourally for various stimulus combinations. The animals weighed between 59 and 1134g. ▲, vertical gratings against grey; +, horizontal gratings against grey; X, oblique gratings against grey; ■, vertical gratings against horizontal gratings. Data from Muntz and Gwyther (1988a, 1989).

In the case of *Octopus* we have no behavioural evidence on its visual sensitivity, although presumably it is considerably better than that of *Nautilus*. The minimum separable visual acuity of 5' for *Octopus* is comparable to that of fishes and aquatic mammals (Table 1). Nevertheless, it is not clear why the acuity is not even better than this. The retinal mosaic is rather finer than necessary for the acuity that is in fact achieved (Muntz and Gwyther, 1988a), and the pupil size is large enough that diffraction will not be limiting. In *Eledone cirrhosa* (Lamarck), another octopod, spherical aberration is far less than would be needed to limit acuity to this level (Sroczyński and Muntz, 1985). Furthermore, terrestrial animals can achieve much better acuities; in the case of humans, for example, the minimum separable lies between 0.5' and 1' (e.g. Senders, 1948), and in the American kestrel, *Falco sparverius* Linné, the acuity, measured behaviourally using square wave gratings, is 0.19' (Fox *et al.*, 1976). Since, however, the minimum separable acuity, measured behaviourally, has been found to be about 5' for all aquatic animals where it has been measured, it could be the environment itself that is limiting. While not very many data are available on the subject, it is clear that high spatial frequencies are particularly heavily attenuated by the water body itself, and it could be that the ability to resolve very fine detail is consequently irrelevant (see Muntz, 1990 for further discussion).

The ability of octopuses and other coleoid cephalopods to discriminate the plane of polarisation of light is usually attributed to the regular rectilinear array of the microvilli of their receptors (Moody and Parriss, 1961). *Nautilus* lacks such a rectilinear array. Nevertheless, the microvilli within any given bundle remain parallel to each other, and so plane polarised light should still be able to affect the receptors differentially, even though there would be no precise relationship between the plane of polarisation and the receptors stimulated. It would be interesting to know whether *Nautilus* can show any differential response to polarised light.

LITERATURE CITED

Baerends, G. P., B. E. Bennema and A. A. Vogelzang. 1960. Über die Anderung der Sehscharfe mit dem Wachstum bei *Aequidens portalegrensis* (Hensel) (Pisces, Cichlidae). *Zoologische Jahrbücher* 88:67-78.

Barber, V. C. and D. E. Wright. 1967. The fine structure of the sense organs of the cephalopod mollusc *Nautilus. Zeitschrift für Zellforschung und micoscoskopichse Anatomie* 102:293-312.

Brunner, G. 1934. Über die Sehschärfe der Elritze *(Phoxinus laevis)* bei verschieden Helligkeiten. *Zeitschrift für vergleichende Physiologie* 21:296-316.

Carlson, B. A., J. N. McKibben and M. V. DeGruy. 1984. Telemetric investigation of vertical migration of *Nautilus belauensis* in Palau (Western Caroline Islands, Pacific Ocean). *Pacific Science* 38:183-188.

Clarke, G. L. and E. J. Denton. 1962. Light and animal life. *In: The Sea*, Vol. 1, M. N. Hill, ed. pp. 456-468. John Wiley, New York.

Dartnall, H. J. A. 1953. The interpretation of spectral sensitivity curves. *British Medical Bulletin* 9:24-30.

Darwin, C. 1958. *The Origin of Species.* Reprint of 1st edition (1859), J. W. Burrow, ed. Penguin Books, Harmonsworth. 477 pp.

Donovan, D. T. 1977. *In: Symposium on the biology of cephalopods,* M. Nixon and J. B. Messenger, eds. *Symposia of the Zoological Society of London* 38:277-285.

Fox, R., S. W. Lehmkuhle and D. H. Westendorf. 1976. Falcon visual acuity. *Science* 192:263-265.

Hartline, P. H., A. C. Hurley and G. D. Lange. 1979. Eye stabilization by statocyst mediated oculomotor reflex in *Nautilus. Journal of Comparative Physiology* 132:117-1226.

Hurley, A. C., G. D. Lange and P. H. Hartline. 1978. The adjustable "pinhole camera" eye of *Nautilus. Journal of Experimental Zoology* 205:37-44.

Jander, R., K. Daumer and T. H. Waterman. 1963. Polarised light orientation by two Hawaiian decapod cephalopods. *Zeitschrift für vergleichende Physiologie* 46:383-394.

Messenger, J. B. 1981. Comparative physiology of vision in molluscs. *In: Handbook of Sensory Physiology,* vol. VII/6C, *Comparative physiology and evolution of vision in invertebrates*, H. Autrum, ed. pp. 93-200. Springer-Verlag: Berlin, Heidelburg, New York.

Moody, M. F. and J. R. Parriss. 1961. The discrimination of polarised light by *Octopus:* A behavioural and morphological study. *Zeitschrift für vergleichende Physiologie* 44:269-291.

Morton, J. E. 1967. *Molluscs* (4th edition). London. Hutchinson. 244 pp.

Muntz, W. R. A. 1977. Pupillary responses of cephalopods. *In: Symposium on the biology of cephalopods,* M. Nixon and J. B. Messenger, eds. *Symposia of the Zoological Society of London* 38:277-285.

Muntz, W. R. A. 1986. The spectral sensitivity of *Nautilus pompilius. Journal of Experimental Biology* 126:513-517.

Muntz, W. R. A. 1987. Visual behaviour and visual sensitivity of *Nautilus pompilius. In: The biology and paleobiology of a living fossil.* W. B. Saunders and N. H. Landman, eds. pp. 231-244. Plenum Press, New York.

Muntz, W. R. A. (1990). Stimulus, environment and vision in fishes. *In: The visual system of fish.* R. H. Douglas and M. B. A. Djamgoz, eds. pp. 491-511. Chapman and Hall, London.

Muntz, W. R. A. and J. Gwyther. 1988a. Visual acuity in *Octopus pallidus* and *Octopus australis. Journal of Experimental Biology* 134:119-129.

Muntz, W. R. A. and J. Gwyther. 1988b. Visual discrimination of distance by octopuses. *Journal of Experimental Biology* 140:345-355.

Muntz, W. R. A. and J. Gwyther. 1989. The visual acuity of octopuses for gratings of different orientations. *Journal of Experimental Biology* 142:461-464.

Muntz, W. R. A. and U. Raj. 1984. On the visual system of *Nautilus pompilius. Journal of Experimental Biology* 109:253-263.

Muntz, W. R. A. and S. L. Wentworth. 1987. An anatomical study of the retina of *Nautilus pompilius. Biological Bulletin* 173:387-397.

Nakamura, E. L. (1968). Visual acuity of two tunas, *Katsuwonus pelamis* and *Euthyunus affinis. Copeia* 1:41-49.

Packard, A. (1969). Visual acuity and eye growth in *Octopus vulgaris* (Lamarck). *Monitore Zoologica Italiana (N.S.)* 3:19-32.

Schusterman, R. J. and R. F. Balliet. 1970. Visual acuity of the harbour seal and the stellar sea lion underwater. *Nature, London* 226:563-564.

Senders, V. L. (1948). The physiological basis of visual acuity. *Psychological Bulletin* 45:465-490.

Sivak, J. G. 1982. Optical properties of a cephalopod eye. *Journal of Comparative Physiology* 147A:323-327.

Sroczyński, S. and W. R. A. Muntz. (1985). Image structure in *Eledone cirrhosa*, an octopus. *Zoologische Jahrbüker* 89:157-168.

Sutherland, N. S. (1963). Visual acuity and disrimination of stripe widths in *Octopus vulgaris* Lamarck. *Pubblicationes Stazione Zoologica Napoli* 33:92-109.

Teichert, C. and T. Matsumoto. 1987. The ancestry of the genus *Nautilus. In: Nautilus: The Biology and Paleobiology of a Living Fossil.* W. B. Saunders and N. H. Landman, eds. pp. 25-32. Plenum Press, New York.

Vanfleteren, J. R. 1982. Monophyletic line in evolution? Ciliary induced receptor membranes. *In: Visual Cells in Evolution.* J. A. Westfall, ed. pp. 107-136. Raven Press, New York.

Ward, P. D., B. Carlson, M. Weekley and B. Brumbaugh. 1984. Remote telemetry of daily vertical and horizontal movement by *Nautilus* in Palau. *Nature, London* 309:248-250.

Wells, M. J. 1978. *Octopus: Physiology and Behaviour of an Advanced Invertebrate.* London: Chapman and Hall. 417 pp.

White, G. M. 1924. Reactions of the larve of the shrimp, *Palaemonetes vulgaris*, and the squid, *Loligo pealii*, to monochromatic light. *Biological Bulletin* 47:265-273.

Willey, A. 1902. *Contribution to the natural history of the pearly Nautilus: Zoological results based on material from New Britain, New Guinea, Loyalty Islands and elsewhere, collected during the years 1895, 1896 and 1897:* Part 6:691-683. University Press Cambridge, England.

Yamamoto, T. 1985. Ontogeny of the visual system in the cuttlefish, *Sepiella japonica.* I. Morphological differentiation of the visual cell. *Journal of comparative Neurology* 232:347-361.

Yamamoto, T., K. Tasaki, Y. Sugarara and A. Tonosaki. 1965. Fine structure of the octopus retina. *Journal of Cell Biology* 25:345-365.

Yamanouchi, T. 1956. The visual acuity of the coral fish *Microanthus strigatus* (Cuvier and Valenciennes). *Publications of the Seto Marine Biology Laboratories* 5:133-156.

Young, J. Z. 1962a. The retina of cephalopods and its degeneration after optic nerve section. *Philosophical Transactions of the Royal Society of London* B245:1-18.

Young, J. Z. 1962b. The optic lobes of *Octopus vulgaris. Philosophical Transactions of the Royal Society of London* B245:19-58.

Young, J. Z. 1971. *The Anatomy of the Nervous System of Octopus vulgaris.* Oxford University Press. 690 pp.

Date of manuscript acceptance: 9 November 1990

Complex learning in *Octopus bimaculoides*

Jean Boal

Curriculum in Ecology, University of North Carolina, Chapel Hill, North Carolina, 27599-3275, U.S.A.

Abstract. In order to investigate complex learning in *Octopus bimaculoides* (Pickford and McConnaughey), I presented subjects with a series of combinations of mollusc shells. Combinations consisted of two shells of one type and an odd shell of another type. The shells were suspended in the octopuses' home tanks, and the animals were rewarded with food for correctly grabbing the odd shell. Associative learning was demonstrated by the subjects' eventual mastery (70 - 100% success rates) of each combination in a series (A+ B− B−), (C+ D− D−) ... By mastery of new combinations of the same stimuli, (A+ D− D−), (C+ B− B−) ,..., subjects demonstrated transfer of learning. Learning improved across successive combinations, evidence for learning set formation. However, because octopuses did not learn to choose the odd stimulus when trained only with non-repeating combinations, no evidence indicated that the octopuses formed the relative class concept of oddity.

The demonstration of complex learning in cephalopods could provide important insight into the evolution of cognition. Most research on complex learning has focused on higher vertebrates. However, an ecological approach suggests that complex learning could evolve whenever it was adaptively advantageous (Shettleworth, 1984). Several investigations have suggested a connection between complex learning abilities and sociality (see Humphrey, 1976; Essock-Vitale and Seyfarth, 1986). Wells (1978) has argued, however, that because the predominantly solitary octopuses have no obvious means of self defense and live in a highly competitive environment, they also could show complex learning. The experiments reported here test this proposal.

I presented *Octopus bimaculoides* (Pickford and McConnaughey) with three objects, two alike and one different, and rewarded them with small pieces of squid if they grabbed the odd one. Using this methodology, I could pose a series of problems differing in the complexity of learning required for successful mastery.

The first question was, could the octopuses learn to choose a particular shell, in repeated presentations of the same combination? Simple associative learning of this sort has previously been demonstrated with *Octopus vulgaris* (Cuvier) (Wells, 1978).

The second question was, could the octopuses still pick the correct shell if known shells were arranged into new combinations? Positive results would show transfer of learned response tendencies.

Thirdly, as the animals gained experience in learning shell combinations, would they improve at learning new but similar tasks? An ability for learning to learn, or developing a learning set, has not been shown previously in an invertebrate, although the related task of learning reversals has (Mackintosh, 1965; Morrow and Smithson, 1969).

Lastly, could the octopuses eventually generalize and immediately choose the odd shell when presented with new combinations? Generalization has been shown in tactile discriminations for *Octopus vulgaris* (Wells and Young, 1970). This task is particulary significant because oddity is an abstract concept, defined only in relationship to other objects and not by any attribute of the object itself. So far, only higher invertebrates have shown evidence of forming such relative class concepts (Thomas, 1980; Lombardi *et al.*, 1984; Thomas and Noble, 1988).

METHODS

EXPERIMENT I

Subjects were three wild caught adults of undetermined sex which had been living in the laboratory a full six months before experiments began. In the laboratory, they were nocturnal and not easy to clock-shift. Therefore, experiments were performed at night under red light.

The octopuses were trained initially to take small pieces of frozen squid from a rod and, later, to grab a single plastic triangle or square on the end of a rod to get a food reward. On days when they were unsuccessful with discrimination tasks, I fed them after trials using the rod alone, up to their minimum daily intake.

Trials consisted of presenting three stimuli, two alike and one different. Six different combinations were used (Appendix 1). The combinations presented were composed of mollusc shells varying in color, texure, and shape, except for combinations four and five, which consisted of plastic shapes which varied in both texture and shape.

I suspended the stimuli on nylon monofilament in the

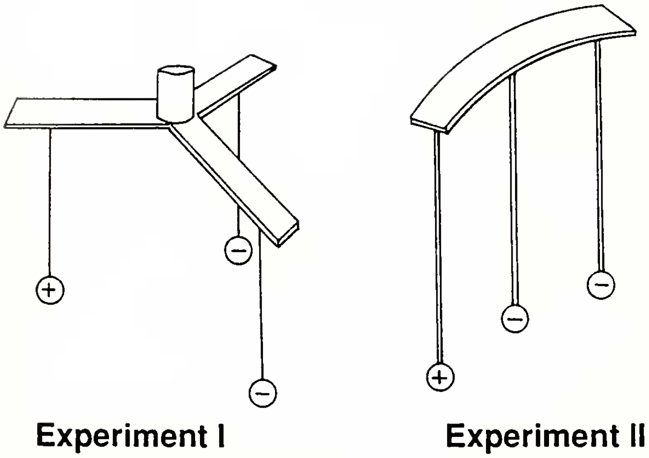

Fig. 1. Apparatus for presenting combinations of shells. Location of the odd, positive stimulus (+) was determined randomly. In Experiment I, the shells were suspended on monofilament; in Experiment II, they were attached to acrylic rods.

octopuses' tanks (Fig. 1). The location of the odd object was determined randomly, with the constraint that in half of the presentations it was in the front half of the tank, and in half it was in the back. A subject was then given two minutes in which to grab one of the stimuli. Responses were usually immediate. A correct response was promptly rewarded with a small piece of squid. I gave each subject eight trials per learning session, ten to fifteen minutes apart, with two learning sessions per day, 11 sessions per week.

In Experiment I, each of the six combinations was presented for 11 sessions or until all subjects reached a success rate of greater than 50% for three successive sessions. The second combination was cut short because one of the shells was shattered by a particularly vigorous grab. Octopuses were then retested for two or three sessions with each familiar combination. They were then each given an equal number of presentations with three of eight arbitrarily chosen new combinations of the same, familiar stimuli. Positive, rewarded shells remained positive and negative, unrewarded shells remained negative; however, the particular combinations of positive and negative stimuli were new.

Experiment I ran for a total of 119 sessions across 12 weeks. Response rates averaged 43% for the three octopuses. For each octopus, sessions with fewer than two grabs were eliminated from the study.

EXPERIMENT II

Subjects were three freshly caught *Octopus bimaculoides*, just reaching sexual maturity, one female and two males. Initial training was carried out as described above. Trials consisted of presentation of three stimuli, two alike and one different, as before. All stimuli used were mollusc shells (Appendix 2). Shells were presented in a line (Fig. 1) to correct for the location bias found in Experiment I. I gave the octopuses ten trials each learning session, spaced five to ten minutes apart, with one session per day, six days per week.

In this experiment, every combination presented to a subject (each trial) was novel. Sixty pairs of shells were used, with every type of shell presented both as a positively rewarded odd shell and as a negative pair, in order to control for the possibility that subjects had attended to some attribute other than oddity. Each type of shell was therefore seen by the octopuses only twice within each week: once as a positive single shell, and once as a negative pair of shells. The only way to solve this problem successfully would be to employ the relative class concept of oddity.

Combinations of shells were determined randomly with the constraint that positive odd shells differed from the negative pair by two of the three features of color, texture, or type (bivalve or gastropod) (Appendix 2). Orders of presentation and locations of the odd shells were randomized.

Simple reinforcement trials were given at the beginning and end of each training session and randomly interspersed among the oddity discrimination problems. These trials consisted of presenting the octopus with the single plastic triangle or square used in initial training. Octopuses were rewarded for grabbing the shape (no choice or discrimination was involved). Response rates in discrimination tasks were 89%. To reduce any inadvertent cuing, beginning with the second week of trials a gauze curtain was draped between the experimenter and the octopuses, with red lights only on the octopuses' side.

RESULTS

EXPERIMENT I

Performances of the three octopuses were statistically indistinguishable (contingency table for three subjects versus correct or incorrect response: $\chi^2 = 0.694$, d.f. = 2, $P > 0.70$). Results were therefore pooled.

Octopuses showed a strong bias towards objects in the front of the tank in the first experiment ($\chi^2 = 107.15$, d.f. = 1, $P < 0.01$); subjects grabbed front stimuli more frequently and less accurately. Response rates were low and not related to success rates (Figs. 2, 3).

Could the octopuses learn to choose correctly one object out of a combination with repeated presentations? Success rates for the first combination, in terms of correct choices as a percentage of total grabs (Fig. 2), showed clearly that the combination was learned and retained. Despite the small sample size and wide variability in performances, learning curves for the first 11 sessions of all six combinations (Fig. 3) demonstrated that the octopuses were able to learn to choose the correct object consistently.

Could they still choose the correct shell if known shells were recombined in new ways? This task required the ability

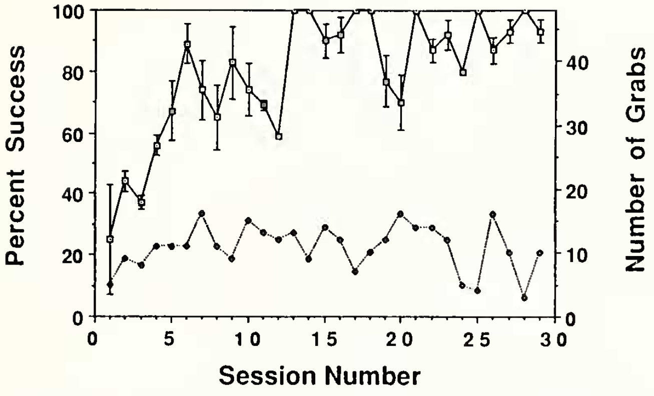

Fig. 2. Mean success rates (squares) (number of correct grabs as percentage of total grabs) with standard errors and total number of grabs (diamonds) across sessions, for the three octopuses on the first discrimination combination.

to remember five (combination two had been broken) simultaneous discriminations, or positive or negative attributes of at least five of the ten different stimuli. Their high success rate with the familiar arrangements shows good retention over this time period, and also shows that five simultaneous discriminations can be mastered by these octopuses. That they performed equally well on the original and new combinations (t = 1.01, d.f. = 26, P > 0.20) is evidence of transfer of learning (Fig. 4); they could use the learned information about member shells of a combination in a new context.

Could the octopuses improve at the task of learning new combinations? Variability was too high and sample sizes too small for comparisons to be made among slopes and intercepts of the six learning curves in the series. However, performances on days one and five (Fig. 5) (or for combination two, the last two completed learning sessions) across the six sequential combinations showed a trend toward improvement in first-day performances. For the sixth combination, a second observer was present on the first day and not on the fifth, a difference that could have affected performances. A paired comparisons test on all six combinations for the two days showed significant effects for both day and combination (days, F = 18.98, d.f. = 1,5, P < 0.01; combinations, F = 10.81, d.f. = 5,5, P < 0.025). The octopuses thus both learned the individual combinations and improved across the series. Sample sizes were too small to control for any effects of shells (combinations 1-3, and 6) versus plastic objects (combinations 4-5).

The last question was, could the octopuses generalize and choose the odd object after learning a number of different combinations? Their very high success rates on day one for the later combinations suggested that they might have formed the relative class concept of oddity. Experiment II was undertaken to explore this possibility further.

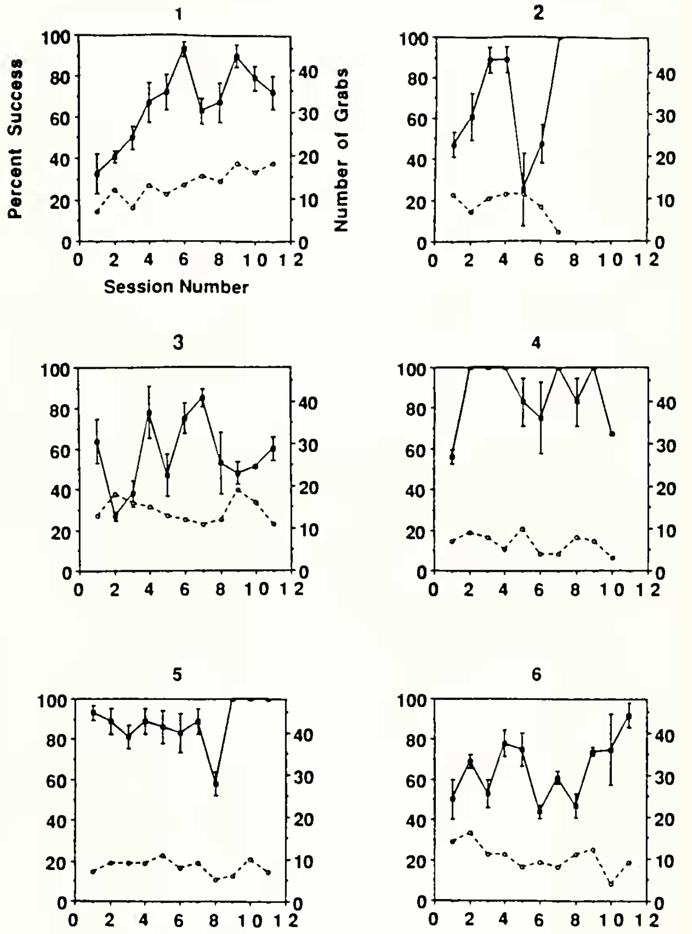

Fig. 3. Mean success rates and standard errors for each of the six combinations (n=3). Dotted lines indicate the total number of responses. Note the above-random performance on all combinations, despite wide variation.

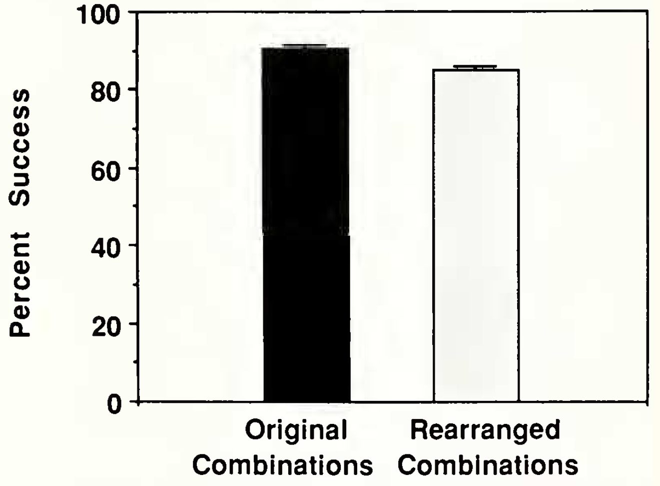

Fig. 4. Mean success rates and standard errors for the three octopuses with five original combinations (n=13) and with eight new combinations of familiar stimuli (n=15).

EXPERIMENT II

Performances of the three octopuses were once again statistically indistinguishable (contingency table for three subjects versus correct or incorrect response: $\chi^2 = 0.132$, d.f. $= 2$, $P > 0.90$). Results were therefore pooled.

The performance of the octopuses in choosing the odd object showed no clear improvement over time (Fig. 6). When mean success rates are separated by week (or by natural breaks), performances appeared slightly, but not significantly, better than completely random (33%). The means by week were 38, 36, 36, 47, and 40% (n=18).

The periodicity found in this learning curve (Fig. 6) was unexpected. It did not correspond to the six session work weeks and was not evident in all three individuals' performances (Fig. 7).

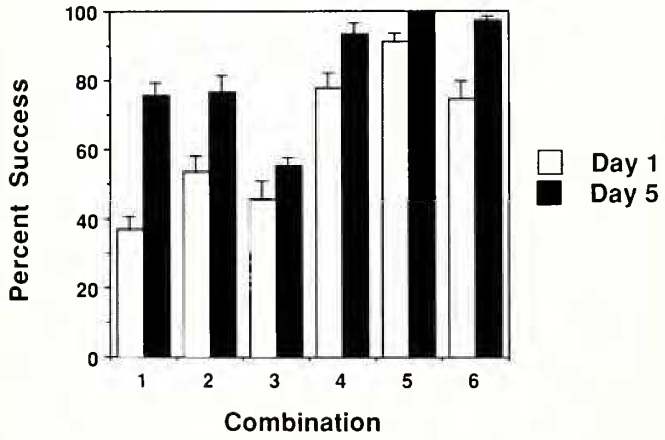

Fig. 5. Mean success rates and standard errors for days one and five for each of the six sequential combinations.

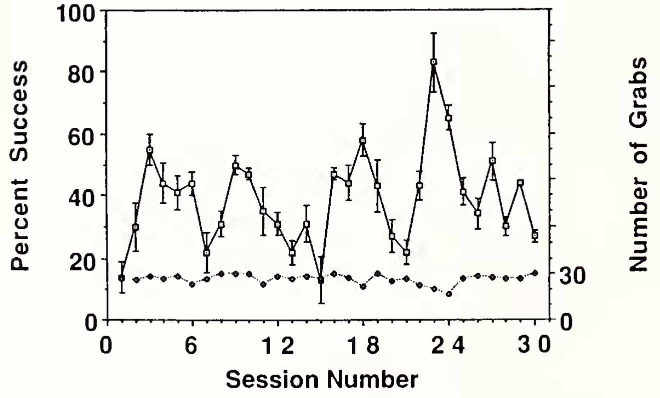

Fig. 6. Mean success rates (squares) with standard errors (n=3) and total numbers of responses (diamonds) across sessions in Experiment II when every combination presented was novel.

DISCUSSION

Octopus bimaculoides, a small octopus from the southern California coast, is a solitary, nocturnal predator, feeding primarily on gastropods but also on bivalves, polychaete worms, fishes, and crabs (Forsythe *et al.*, 1984). It lives in dens or burrows and interacts rarely with other octopuses (Lang, 1990). Generations do not overlap, which precludes the level of social learning available to even the most solitary of mammals. Laboratory-reared octopuses have the reputation of being slower to learn than those caught wild, perhaps an indication that learning takes place normally in their environment.

The particular tasks in these experiments do not relate directly to any activities known to be performed in the wild. However, they are comparable to experimental tests used to assess cognitive abilities in a wide range of species (Thomas, 1980).

In Experiment I, the octopuses' mastery of the simple task of learning to choose a particular shell after repeated presentations of the same combination is clear evidence of associative learning. This result is consistent with findings for *Octopus vulgaris* (Wells, 1978) as well as other invertebrates (Corning *et al.*, 1976).

Transfer of learning was also evident from performances on new combinations of familiar stimuli, an indication that their learning was not tightly dependent on context. Whether they based their choices on learned positive identities, learned negative identities, or both, cannot be determined from this experiment. However, response patterns suggest that they did in fact learn both. The octopuses normally sat in one of the top front corners of the tanks during trials. During the first experiment, they quickly stopped swimming to the far side of the tank (approximately an extra 20 cm) in order to grab the far stimulus. Therefore, they had to decide not only which stimulus to grab, but which stimuli not to grab, since the two nearby shells might both be negatives. They were highly successful at learning these discriminations, an indication that they had, in fact, learned both positive and negative shell identities.

That learning was retained over time was shown in the transfer of learning trials. The octopuses' success rate with the first combination was 80%, when retested after five weeks of experience with other combinations. Informal observations suggest that these animals might show retention even for months.

Learning set formation, or learning to learn, implies that something beyond recognition of objects has been learned. It could be only to attend carefully to relevant stimuli. Or, it could involve remembering previous choices and outcomes so as to arrive more quickly at correct solutions. Although learning set formation has not been shown previously in invertebrates, the related task of learning reversals has been mastered by isopods (Morrow and Smithson, 1969) and

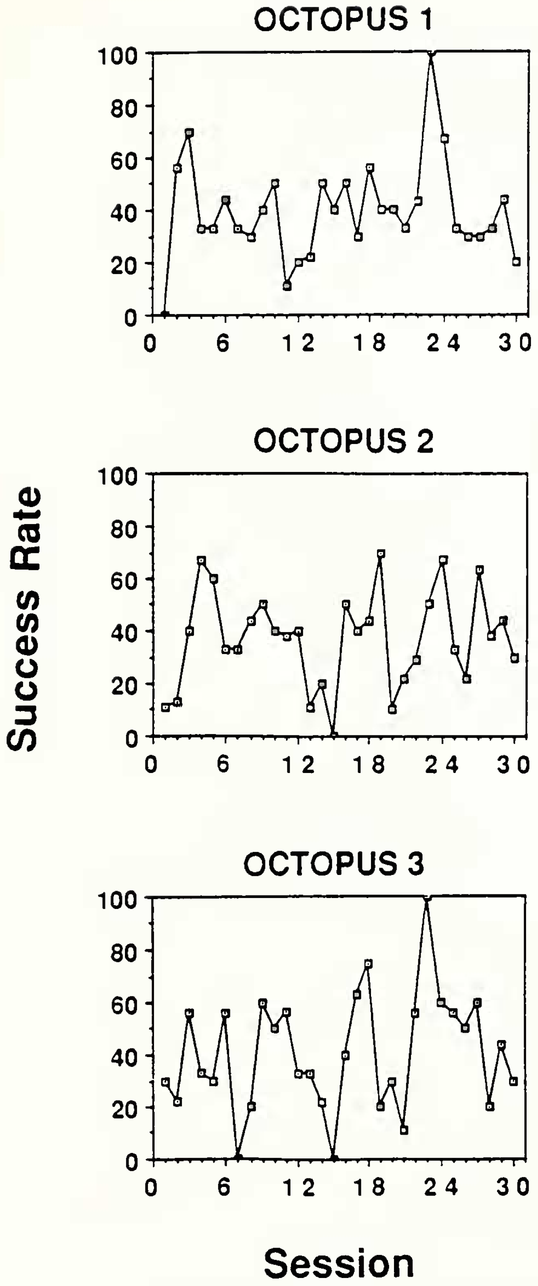

Fig. 7. Mean success rates across sessions for each subject in Experiment II when every combination presented was novel.

by octopuses (Mackintosh, 1965). Now that learning set formation has been indicated in octopuses, experiments with a longer learning set (many more combinations) could make comparisons with vertebrate species possible.

The results from the Experiment II showed no evidence of octopuses learning the concept of oddity. The results were surprising, after the octopuses' promising first day performances towards the end of the learning set. It is possible that more time was needed; the first experiment took place across three months as opposed to only five weeks for the second experiment. Or, it could be that repeated learning of exemplars, as in the first experiment, could succeed where the repeated novel presentations did not. It also could be that the curtain used during the second experiment prevented cuing that biased the first experiment.

The apparent periodicity in the learning curve for this experiment has no clear explanation. The learning curves for the first experiment also showed temporal variability in performance, especially prior to mastery of the task, but no apparent periodicities. Sanders (1977) has documented octopus learning curves to be multiphasic, corresponding to possible transitions between short- and long-term memory processes. However, his experiments were examining retention of a learned task across hours as opposed to performances of new tasks across days. I expect that the pattern observed in this experiment was simply an artifact of the small sample size and short duration of the experiment.

There were a number of problems in Experiment I. First, response rates were low, perhaps because, with so many trials each day, the smallest practical food rewards still added up to more than their normal daily intake levels. Another related explanation is that older, laboratory-habituated animals seem to have smaller appetites and to be less responsive in general. Second, choices during the first trial of each training session were significantly less accurate, as compared to subsequent trials ($\chi^2 = 22.884$, d.f. $= 7$, $P < 0.01$). Results from this experiment were therefore conservative. Third, animals were significantly more likely to grab stimuli in the front half of the tank. The experimental design was balanced, however, for front and back placement of the odd object. These three problems appeared to be addressed successfully in Experiment II by switching to a linear presentation, providing simple reinforcement trials at the beginning of each session, limiting trials to ten per day, and using younger, freshly caught animals.

Clearly, octopuses are capable of some forms of complex learning. While it remains to be seen if these octopuses can master a relative class concept such as oddity, these exploratory experiments suggest that the abilities underlying the formation of a learning set have evolved in an invertebrate, as well as in vertebrates (Pearce, 1987). They provide further evidence for convergences in function despite divergences in physiology between invertebrates and vertebrates (Packard, 1972; Corning *et al.*, 1976).

ACKNOWLEDGMENTS

I would like to thank W. M. Kier for sharing his expertise and for use of his laboratory, R. H. Wiley for his encouragement and thoughtful critiques, and A. Smith for his camaraderie in times of aquatic crisis. I would also like to thank my anonymous reviewers for their helpful comments. This research was supported by the University of North Carolina Curriculum in Ecology and Sigma Xi Grants-in-Aid of Research.

LITERATURE CITED

Corning, W. C., J. A. Dyal and R. Lahue. 1976. Intelligence: an invertebrate perspective. *In: Evolution, Brain and Behavior: Persistent Problems.* R. B. Masterson, W. Hodos, H. Jerison, eds. pp. 215-263. John Wiley & Sons, New York.

Essock-Vitale, S. and R. M. Seyfarth. 1986. Intelligence and social cognition. *In: Primate Societies.* B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham, T. T. Struhsaker, eds. pp. 452-461. University of Chicago Press, Chicago.

Forsythe, J. W., R. H. DeRusha and R. T. Hanlon. 1984. Notes on the laboratory culture of *Octopus bimaculoides,* the California mudflat octopus. *American Malacological Bulletin* 2:92.

Humphrey, N. K. 1976. The social function of intellect. *In: Growing Points in Ethology,* P. P. B. Bateson and R. A. Hinde, eds. pp. 303-317. Cambridge University Press, New York.

Lang, M. A. 1990. Population dynamics and life history of *Octopus bimaculoides.* Abstract. American Malacological Union, 56th Annual Meeting Program, p. 47.

Lombardi, C. M., C. C. Fachinelli and J. D. Delius. 1984. Oddity of visual patterns conceptualized by pigeons. *Animal Learning and Behavior* 12(1):2-6.

Mackintosh, N. J. 1965. Discrimination learning in the octopus. *Animal Behavior* Supplement 1, 129-134.

Morrow, J. E. and E. L. Smithson. 1969. Learning sets in an invertebrate. *Science* 164:850-851.

Packard, A. 1972. Cephalopods and fish: the limits of convergence. *Biological Reviews* 47:241-307.

Pearce, J. M. 1987. *An Introduction to Animal Cognition.* Lawrence Erlbaum Associates, London. 328 pp.

Sanders, G. D. 1977. Multiphasic retention performance curves: fear or memory? *Symposia of the Zoological Society of London* 38:435-445.

Shettleworth, S. J. 1984. Learning and behavioral ecology. *In: Behavioral Ecology: An Evolutionary Approach.* J. R. Krebs, N. B. Davies, eds. pp. 170-194. Sinauer, Sunderland, Massachusetts.

Thomas, R. K. 1980. Evolution of intelligence: an approach to its assessment. *Brain, Behavior and Evolution* 17:454-472.

Thomas, R. K. and L. M. Noble. 1988. Visual and olfactory oddity learning in rats: what evidence is needed to show conceptual behavior? *Animal Learning and Behavior* 49(3):395-409.

Wells, M. J. 1978. *Octopus: Physiology and Behavior of an Advanced Invertebrate.* Chapman and Hall, London. 417 pp.

Wells, M. J. and J. Z. Young. 1970. Stimulus generalization in the tactile system of *Octopus. Journal of Neurobiology* 2(1):31-46.

Date of manuscript acceptance: 5 November 1990

APPENDIX 1

Combinations in Experiment I

Combination 1: *Noetia ponderosa* (Say) (+), *Anomia simplex* Orbigny (−).

Combination 2: *Anomia simplex* Orbigny (+), *Chione cancellata* (Linné) (−).

Combination 3: *Aequipecten gibbus* (Linné) (+), *Mercenaria mercenaria* (Linné) (−).

Combination 4: White square plastic grid (3 x 3 x 1 cm) (+), white square flat plastic chip (3 x 4 x .4 cm) (−).

Combination 5: white PVC adaptor fitting, threaded by slip, six-sided middle section (2.9 x 4 cm) (+), white PVC pipe section (4.1 cm diam. x 1.6 cm width) (−).

Combination 6: *Crassostrea virginica* (Gmelin) (+), *Busycon contrarium* (Conrad) (−).

APPENDIX 2

Combinations in Experiment II

Trials in Experiment II consisted of presenting combinations of three shells, two of one species and one of another. Species were chosen randomly with the constraint that the two types of shells of a combination must differ on two of three features (texture: 1=smooth, 2=ridged, 3=pointed; type: 1=gastropod, 2=bivalve or slipper; color: 1=light, 2=dark, 3=patterned).

Species included were: *Aequipectin gibbus* (Linné) (2,2,3); *Amphidromus entobaptus* (Dohrn) (1,1,1); *Anadara brasiliana* (Lamarck) (2,2,1); *A. ovalis* (Bruguiere) (2,2,1); *A. ovalis* (Brugiere) (2,2,1); *Anomia simplex* Orbigny (1,2,1); *A. simplex* (1,2,1); *A. simplex* (1,2,2); *Argopecten* sp. (2,2,1); *A.* sp. (2,2,2); *A.* sp. (3,2,3); *A.* sp. (2,2,3); *Arca zebra* Swainson (2,2,3); *Architectonia nobilis* Roding (2,1,3); *Babylonia areolata* (Link) (1,1,3); *Bursa* sp. (3,1,3); *Chicoreus cichoreum* (Gmelin) (3,1,3); *Chione cancellata* (Linné) (2,2,3); *C. cancellata* (2,2,1); *C. cancellata* (2,2,1); *C. cancellata* (2,2,1); *C. paphia Linné* (2,2,3); *Conus pulcher* Lightfoot (1,1,1); *Crassostrea virginica* (Gmelin) (1,2,2); *C. virginica* (2,2,1); *C. virginica* (1,2,2); *C. virginica* (2,2,1); *Crepidula fornicata* (Linné) (1,2,1); *C. fornicata* (1,2,1); *C. fornicata* (1,2,2); *C. fornicata* (1,2,1); *Dodinia discus* (Reeve) (1,2,1); *Ficus subintermedia* (Orbigny) (1,1,1); *Geukensia demissa* (Dillwyn) (2,2,2); *Helicostyla* sp. (1,1,3); *Haliotis asinia* Linné(1,2,2); *Liguus virineus* (Linné) (2,1,3); *Lucina pectinata* (Gmelin) (2,2,1); *Marisa cronuarietis* (Linné) (1,1,3); *Melongena corona* Gmelin (3,1,3); *Mercenaria mercenaria* (Linné) (1,2,1); *Murex fulvescens* Sowerby (3,1,1); *Natica* sp. (1,1,1); *N. stellata* Chenu (1,1,1); *Noetia ponderosa* (Say) (2,2,1); *N. ponderosa* (2,2,1); *N. ponderosa* (2,2,2); *Oliva sayana* Ravenel (1,1,1); *O. servicea* Roding (1,1,3); *Phalium granulatum* (Born) (1,1,3); *Pitar morrhuana* (Linsley) (1,2,2); *Pleuroplaca* sp. cf. *glabra* (Dunker) (2,1,1); *Sinum perspectiuum* (Say) (1,2,1); *Sisula solidissima* (Dillwyn) (1,2,1); *Tagelus plebius* (Lightfoot) (1,2,1); *Tectarius* cf. *Coronatus valenciennes* Gmelin (3,1,3); *Telescopium telescopium* (Linné) (1,1,2); *Tellina alternata* Say (2,2,1); *Trachycardium egmontium* (Shuttleworth) (2,2,1); *T. egmontium* (2,2,2); *Turbo* sp. (1,1,1); *T.* sp. cf. *petholatus* Linné (1,1,2); *Turritella* sp. (2,1,3); *Vexillum rugosum* (Gmelin) (2,1,3).

Mating behavior of the freshwater pulmonate snail, *Physa gyrina*

Thomas J. DeWitt

Department of Biology, State University of New York, Binghamton, New York 13902-6000, U.S.A.

Abstract. The typical mating sequence of freshwater pulmonate snails includes mounting, positioning, preputium eversion, intromission and dismounting. Previous studies report that sperm recipient behavior is passive or absent, and fecundity increases with size. I examined the mating sequence, mate rejection and fecundity of *Physa gyrina* (Say) removed from the Fenway in Boston, Massachusetts. Mate rejection occurred in any of four stereotypical ways: 1) shell swinging; 2) shell jerking; 3) increasing distance to the gonopore; 4) making head/preputium contact. The jerking behavior corresponds to what others described as sham copulation. Shell swinging was similar to parasite escape behavior. Fecundity increased with the size of sperm recipients but decreased with the size of sperm donors. Differences in methodology could explain discrepancies in the interpretation of mating behavior among studies.

Many studies have been conducted on the reproductive biology of freshwater pulmonate snails (Basommatophora; Geraerts and Joosse, 1984). Several behavioral studies, however, have reported conflicting observations within and among closely related taxa. This variation could be due to genetic variation and phenotypic plasticity, as well as differences in research methodology. The most complete information on basommatophoran reproductive behavior is for the sister families Lymnaeidae and Physidae. Physid snails have been studied recently in terms of predator induced life history shifts (Crowl, 1990; Crowl and Covich, 1990), macrophyte herbivory (Sheldon, 1987), sperm storage (Wethington and Dillon, 1991), parasite defense strategies (Townsend and McCarthy, 1980) and mate choice patterns (T. J. DeWitt, unpub. data).

Physa (=Physella) gyrina (Say) is widely distributed (Clarke, 1981) in lentic environments, especially where pollution or high temperatures exclude other snails (Clampitt, 1970; Harman, 1974). Physid snails, like lymnaeids, are hermaphrodites that usually cross fertilize but are capable of self fertilization (Colton, 1918; R. M. DeWitt and Sloan, 1959). Although the male genital tract can mature briefly before that of the female, it is unclear as to whether physids are ever functionally protandric (Duncan, 1959). Furthermore, snails can receive sperm regardless of the sexual maturity of female tracts, but can not oviposit (Duncan, 1959).

Peak breeding in the field for *Physa gyrina* is reported to occur from April through June in Iowa (Clampitt, 1970) and Michigan (R. M. DeWitt, 1955), although mature snails collected any time of the year will breed in the laboratory as long as water temperature exceeds 10°C (R. M. DeWitt, 1955). This corresponds to the April to May peak reported for *P. fontinalis* Linné by Duncan (1959) at a similar latitude.

In this paper, I report observances taken on the reproductive behavior of *Physa gyrina* removed from the field. I examined the typical mating sequence, mate rejection, and fecundity. Conflicting reports on mating behavior within and between pulmonate snail species could be due to research methodology.

METHODS

On six independent occasions between February and May 1990, 300-500 snails were collected from the same location in the Fenway in Boston, Massachusetts (NW of Beth Israel Hospital). Snails were collected between 1200 and 1600 hours. Dip nets were used to collect litter from the bottom of the Fenway (1-1.5 m depth). Snails were removed from the litter, by hand, to a bucket containing Fenway water. On all occasions, *Physa gyrina* made up over 98% of snail fauna [other species, in order of abundance, included *Planorbella campanulata (=Helisoma campanulatum)* (Say) and *Stagnicola elodes (=Lymnaea palustris)* (Say)]. Each collection, including all specimens, was brought back to the laboratory and placed in an aerated, filtered 38 *l* aquarium with approximately 20 *l* of water (half from the collection site, the other half was conditioned tap water). Population densities were greater than, but similar to those observed in the field during collection. Snails were kept at 20°C near a natural light source. They were fed canned or boiled vegetables (carrot, pea, chick pea). This diet was supplemented by unidentified algae in the aquaria.

Preliminary observations of snails were made to define criteria for successful versus failed copulation. Criteria I used to define successful mating were that 1) the sperm donor was in place at the shell margin of the recipient; 2) the preputium was placed in the immediate area of the gonopore; 3) the seminal vesicle within the preputium contained a milky

American Malacological Bulletin, Vol. 9(1) (1991):81-84

substance; 4) no agonistic behavior occurred within the first 30 seconds of mating.

TREATMENT 1

After approximately 18 hours from the time of collection, interacting *Physa gyrina* pairs were observed. As soon as the nature of each interaction was determined, pairs were removed and measured from the shell apex to the most distal portion of the shell margin. Sperm recipients from successful matings were removed to mason jars for a week and the number of eggs they laid was recorded. This continued until 12 successful matings were observed on each of the six occasions (72 observations total).

TREATMENT 2

An additional 90 *Physa gyrina* pairs were observed as in treatment one. In treatment two, however, pairs were not interrupted, but allowed to finish their interactions.

RESULTS

The sizes of sperm donors and recipients in treatment one were 7.32 ± 0.96 and 7.90 ± 1.12 (mean ± sd), respectively. The generalized mating sequence for *Physa gyrina* was similar to that described for *Lymnaea stagnalis* (Linné) (van Duivenboden and Maat, 1988), a closely related taxon, except that it occurred more rapidly. The sequence of successful mating included mounting (mate selection?), positioning, eversion of the preputium, intromission and dismounting. This process was sometimes interrupted by mate rejection but lasted approximately 10-20 minutes when successful (Fig. 1).

Rejection of mates was observed to occur in four stereotypical ways (Fig. 2). In order of decreasing frequency, intended sperm recipients performed 1) shell swinging, 2) shell jerking, 3) shell positioning such that sperm donors could not reach the gonopore, 4) head to preputium contact (“biting”).

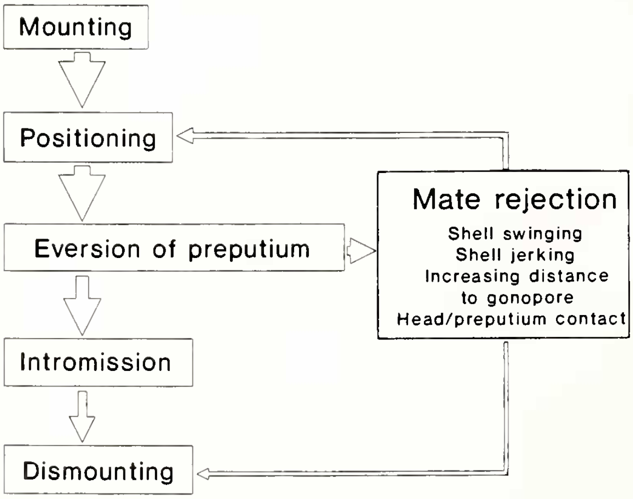

Fig. 1. Mating sequence of *Physa gyrina*. Thickness of arrows approximates the frequency that path is followed.

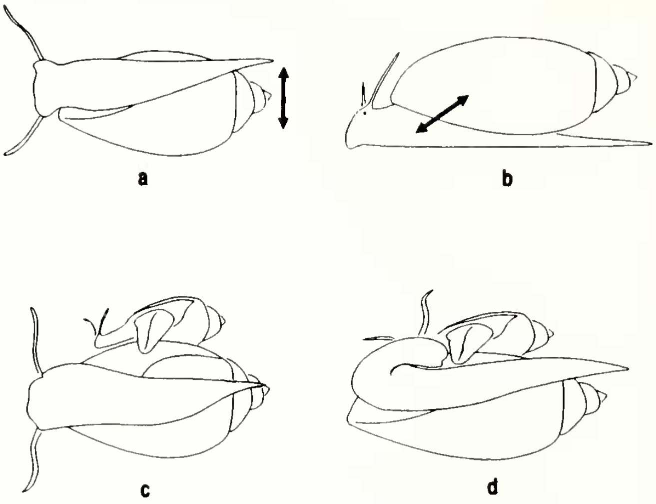

Fig. 2. Stereotypical mate rejection behavior by sperm recipients. a) shell swinging, b) shell jerking, c) increasing distance to gonopore, d) head/preputium contact.

Shell swinging was characterized by a 180-270° left and right twisting of the shell about the stationary foot. Shell jerking was rapid contraction of the shell toward the foot, with gradual release. This was generally repeated several times until the preputium was withdrawn. Shell positioning by intended sperm recipients occurred such that the distance from their shell margin to their gonopore was increased, effectively placing the gonopore out of reach of the sperm donor's preputium. I could not observe whether head to preputium contact included radular action but think it likely given that physid snails rarely cease radular movement and that the reaction from sperm donors was always immediate, violent withdrawal of the preputium, usually followed by immediate dismount. The first three forms of agonistic sperm recipient behavior generally resulted in two or three re-attempts at mating by sperm donors before they dismounted.

I commonly observed chain copulations of up to five snails, acting alternately as sperm donors and recipients. Furthermore, triads were observed on three occasions while collecting in the field. Polyandrous copulation, which has only been clearly described (among basommatophorans) for *Physa fontinalis* (Duncan, 1959), was not observed.

The range of snails observed to oviposit in this study was 6.3-10.9 mm. Larger sperm recipients proved significantly more fecund (R^2 for log transformed data = 0.58, $p_{\alpha/2}$ = 0.0001). However, the size of sperm donors was negatively correlated with sperm recipient (log transformed) fecundity (pearson correlated coefficient = -0.44, $p_{\alpha/2} < 0.01$), even when the size of sperm recipients was controlled for (partial correlation coefficient = -0.34, $p_{\alpha/2}$ = 0.025).

DISCUSSION

Describing the mating behavior of freshwater gastropods can be accomplished by field or laboratory studies. The former may be made difficult by turbid water, detritus, invasiveness of techniques used to locate snails and low probability of finding mating pairs. For these reasons, laboratory investigation is usually employed. This is acceptable if it is reasonable to believe organisms are behaving as they would in the field. Observations from field studies generally concur with laboratory findings (e.g. R. M. DeWitt, 1954b). However, it is likely that the laboratory techniques employed affect the behavior of animals being studied, and account for some of the variation in reported behavior.

R. M. DeWitt (1954a) reported 7 mm as the minimum size at which *Physa gyrina* would oviposit. However, this research of *P. gyrina*, and studies on other physids, suggest that the size at first oviposition varies among populations and species (Duncan, 1959; Clampitt, 1970; McMahon, 1975; Crowl, 1990). This variation could be due to physical variables such as temperature (McMahon, 1975) and plastic life history strategies. *P. virgata* (Gould) (Crowl, 1989; Crowl and Covich, 1989) is induced, by the presence of crayfish (predators), to delay reproduction until larger size is attained. I have observed similarly varied life history patterns in *P. heterostropha* Say in streams with and without goldfish in New York (unpub. data).

R. M. DeWitt (1954a) reported a lack of chain copulations in *Physa gyrina*. However, I observed chain copulation in the laboratory and field. Chain copulations have been reported for laboratory populations of several other freshwater pulmonates (e.g. Barraud, 1957; Duncan, 1959).

My observation of increasing fecundity as a function of snail size concurs with work on several freshwater pulmonates (e.g. R. M. DeWitt, 1954b; de Wit, 1955; Hunter, 1975; McMahon, 1975). However, this is the first report that the size of sperm donors is negatively correlated with the fecundity of sperm recipients. It will be interesting to see if studies employing different methods obtain similar results. The data from the present study do not allow me to address why larger sperm donors could have suppressed sperm recipient fecundity.

Many studies report that females are passive during mating (R. M. DeWitt, 1954a; Duncan, 1959). Van Duivenboden and ter Maat (1988) state "characteristic female mating behavior is absent". I observed several stereotypical female behavior patterns which I interpret to be mate rejection (Fig. 2). Two of these correspond to behavior reported by other investigators. Shell swinging is similar to weak parasite avoidance reported for *Physa fontinalis* in response to chemicals from predatory leeches (Townsend and McCarthy, 1980). Shell jerking is similar to the description of "false coupling" given by Barraud (1957) and "sham copulation" given by van Duivenboden and ter Maat (1988) for *Lymnaea stagnalis*. Barraud (1957) describes false coupling as when the female gonopore remains unoccupied while the preputium is bent around her shell margin, despite contorted retractions of the female. Van Duivenboden and ter Maat describe sham copulation as "characterized by strong withdrawal of the forepart of the female, after which she relaxes again...while the preputium remains in place (under the female shell margin — not in the gonopore)." Barraud (1957) report that males may move away without copulation after this event and that copulation was seldom successful. Van Duivenboden and ter Maat (1988) report that nearly all copulations were eventually successful but "sham copulations occur frequently (≥ 50% of the pairs)".

I believe the phrase "sham copulation" could be a misnomer because the interpretation as mate rejection is strongly supported in this study and that of Barraud (1957). Natural selection should provide strong selection pressure for successful copulatory mechanisms between conspecifics (Ridley, 1983) unless there is a benefit to unsuccessful mating behavior. Let us examine potential costs and benefits of sham (or unsuccessful) copulation. The only benefit I could associate with sham copulation would be the ethological argument that the practice is valuable for future "true" copulation. Noland and Carriker (1946) show that prior experience increases copulatory success in *Lymnaea stagnalis*. However, later studies by Barraud (1957) and van Duivenboden and ter Maat (1988) refute this. Costs of "sham copulation" could go beyond time and energy expenditure. Predation risk may be greater for animals in a pair versus solitary animals [e.g. *Gammarus pulex* (Linné); Ward, 1986]. Thus, the value of sham copulation is dubious. I suspect that the long period of isolation used in many studies made sperm donors less willing to give up copulation attempts, thereby reducing the occurrence of successful mate rejection and delaying or preventing straightforward copulation.

Researchers often use the isolate-unite technique (e.g. Noland and Carriker, 1946; Barraud, 1957; Duncan, 1959; van Duivenboden and ter Maat, 1988) to study the reproductive behavior of gastropods. This protocol is effective for stimulating copulation, but could lead to overlooking phenomena of more realistic situations for field populations (e.g. mate choice). The protocol could introduce artifact problems as well [e.g. sperm deprivation during isolation evenutally leads to initiation of self fertilization (van Duivenboden, 1983), thereby reducing receptivity to mating].

It is probable that a more generalized view of mating behavior in gastropods can be obtained by supplementing the isolate-unite technique with studies using multiple wild-caught snails in as close conditions to their natural environment as possible (i.e. population density, water, temperature, light, etc.). The use of focal pair sampling (perhaps videotaping) would also be more likely to yield valuable details on mating over multiple pair observation (e.g. van Duivenboden and ter Maat, 1988). The suggested techniques allow elucidation of structured mating, mate rejection and other phenomena occurring in wild populations.

ACKNOWLEDGMENTS

This research was made possible by academic support from Dr. Fred A. Wasserman, to whom I am greatly obliged.

LITERATURE CITED

Barraud, E. M. 1957. The copulatory behaviour of the freshwater snail *(Linnaea stagnalis* L.). *British Journal of Animal Behaviour* 5:55-59.

Colton, H. S. 1918. Self fertilization in the air-breathing pond snails. *Biological Bulletin of Woods Hole* 35:48-49.

Clampitt, P. T. 1970. Comparative ecology of the snails, *Physa gyrina* and *P. integra* (Basommatophora: Physidae). *Malacologia* 10:113-151.

Clarke, A. H. 1981. *The Freshwater Mollusks of Canada.* National Museums of Canada, Ottawa, Canada. 446 pp.

Crowl, T. A. 1990. Life-history strategies of a freshwater snail in response to stream performance and predation: balancing conflicting demands. *Oecologia* 84:238-243.

Crowl, T. A. and A. P. Covich. 1990. Predator-induced life-history shifts in a freshwater snail: A chemically mediated and phenotypically plastic response. *Science* 247:949-951.

de Wit, W. F. 1955. The life cycle and some other biological details of the freshwater snail *Physa fontinalis* (L.). *Basteria* 19:36-73.

DeWitt, R. M. 1954a. Reproduction, embryonic development and growth in the pond snail, *Physa gyrina* Say. *Transactions of the American Microscopical Society* 73:124-137.

DeWitt, R. M. 1954b. Reproductive capacity in a pulmonate snail *(Physa gyrina* Say). *American Naturalist* 88:159-164.

DeWitt, R. M. 1955. The ecology and life history of the pond snail, *Physa gyrina. Ecology* 36:40-44.

DeWitt, R. M. and W. C. Sloan. 1959. Reproduction in *Physa pomilia* and *Helisoma duryi. Animal Behaviour* 7:81-84.

Duncan, C. J. 1959. The life cycle and ecology of the freshwater snail *Physa fontinalis* (L.). *Journal of Animal Ecology* 28:97-117.

Geraerts, W. P. M. and J. Joosse. 1984. Freshwater snails (Basommatophora). In: *The Mollusca, Volume 7: Reproduction,* K. M. Wilbur, ed. pp. 141-207. Academic Press, New York.

Harman, W. N. 1974. Snails (Mollusca: Gastropoda). In: *Pollution Ecology of Freshwater Invertebrates,* C. W. Hart, Jr. and S. L. H. Fuller, eds. pp. 274-312. Academic Press, New York.

Hunter, R. D. 1975. Growth, fecundity, and bioenergetics in three populations of *Lymnaea palustris* in upstate New York. *Ecology* 56:50-63.

McMahon, R. F. 1975. Effects of artificially elevated water temperatures on the growth, reproduction and life cycle of a natural population of *Physa virgata* Gould. *Ecology* 56:1167-1175.

Noland, L. E. and M. R. Carriker. 1946. Observations on the biology of the snail *Lymnaea stagnalis appressa* Say during twenty generations in the laboratory culture. *American Midland Naturalist* 36:467-493.

Ridley, M. 1983. *The Explanation of Organic Diversity: the Comparative Method and Adaptations for Mating.* Clarendon Press, Oxford, United Kingdom. 272 pp.

Sheldon, S. P. 1987. The effects of herbivorous snails on submerged macrophyte communities in Minnesota lakes. *Ecology* 68:1920-1931.

Townsend, C. R. and T. K. McCarthy. 1980. On the defence strategy of *Physa fontinalis* (L.), a freshwater pulmonate snail. *Oecologia* 46:75-79.

van Duivenboden, Y. A. 1983. Transfer of semen accelerates the onset of egg-laying in female copulants of the hermaphrodite freshwater snail, *Lymnaea stagnalis. International Journal of Invertebrate Reproduction* 6:249-257.

van Duivenboden, Y. A. and A. ter Maat. 1988. Mating behaviour of *Lymnaea stagnalis. Malacologia* 28:53-64.

Ward, P. I. 1986. A comparative field study of the breeding behaviour of a stream and a pond population of *Gammarus pulex* (Amphipoda). *Oikos* 46:29-36.

Wethington, A. R. and R. T. Dillon, Jr. 1991. Sperm storage and multiple insemination in a natural population of the freshwater snail, *Physa heterostropha. American Malacological Bulletin* 9(1):99-102.

Date of manuscript acceptance: 22 January 1991

Reproductive patterns and seasonal occurrence of the Sea Hare *Aplysia brasiliana* Rang (Gastropoda, Opisthobranchia) at South Padre Island, Texas

Ned E. Strenth[1] **and James E. Blankenship**[2]

[1]Department of Biology, Angelo State University, San Angelo, Texas 76909, U.S.A.
[2]The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

Abstract. Monthly collections of *Aplysia brasiliana* Rang were made at South Padre Island, Texas over an 18 month period. Seasonal changes in distribution of weight classes support the existence of a maximum life cycle of approximately 1 year. Large numbers of juveniles in spring collections are produced by the reproductive activities of overwintering adults or possibly recruitment of larvae from other areas. The spring recruitment is followed by the disappearance from the population by large overwintering specimens. Spring juveniles increase in size and weight by late summer. Early fall collections yielded no specimens. A minor period of secondary recruitment can occur during late fall or early winter. Winter collections, when successful, yielded reduced numbers of large adults.

A number of investigations conducted on naturally occurring aplysiid populations have centered upon duration of life cycles, seasonal changes in size or weight, reproductive activities, and recruitment into the population by recently metamorphosed juveniles (Miller, 1960; Carefoot, 1967; Usuki, 1970; Audesirk, 1979; Sarver, 1979; Gev *et al.*, 1984). Following a review of these studies, Carefoot (1987) concluded that, while considerable variation does exist in some species, most sea hares do, in fact, exhibit an annual life cycle.

This conclusion is consistent with current information for the sea hare *Aplysia brasiliana*, which is common to the Gulf of Mexico (Strenth and Blankenship, 1978a). For Florida populations of *A. brasiliana*, both Krakauer (1969) and Hamilton *et al.* (1982) concluded that this species exhibits a life cycle of approximately one year. Krakauer's (1969) conclusions relative to *A. brasiliana* (as *A. willcoxi* Heilprin) were, however, based upon only a one year series of collections of relatively small sample sizes. The study by Hamilton *et al.* (1982) was based upon large sample sizes but unfortunately was limited to the months of March through June. Krakauer (1969) also characterized the life cycle of *A. brasiliana* in Florida as having "two waves of settling" with a major "spawning period" in late March and early April. Following several years of preliminary field work, this study was undertaken in an effort to clarify the life cycle of *A. brasiliana*.

METHODS

Collections were made on a continuous monthly basis in the south Laguna Madre at South Padre Island, Texas, at depths of < 1 m, from July, 1977 through December, 1978. All known habitats of *Aplysia brasiliana* were established during preliminary field work in 1975 and 1976. While each of these habitats was surveyed during each trip, collection techniques and success varied with the season. Winter and spring collections were most productive during early morning low tides. These low tides trapped nocturnally active specimens in shallow grass flats which are common along the western coastal margin of the island. Recently metamorphosed juveniles were collected in the spring from artificial habitats of broken concrete, brick and stone, which had been deposited near the east abutment of the old causeway. During summer months, swimming specimens were collected with dip nets at night near the lights of fishing piers located along the western side of South Padre Island. Specimens were also found on rocks of the boat slip at the Coast Guard Station and the channel side of the north jetty. Following collection, specimens were weighed to the nearest gram.

RESULTS AND DISCUSSION

DURATION OF LIFE CYCLE

The results (Fig. 1) of this study support the existence of a maximum life cycle of approximately 1 year for *Aplysia brasiliana* at South Padre Island, Texas. Summer collections, such as those of 1977, were generally characterized by the presence of specimens in the 50 to 300 gm weight range. These animals increased in size and weight during the late fall and winter. By March of the following spring (Fig. 1),

American Malacological Bulletin, Vol. 9(1) (1991):85-88

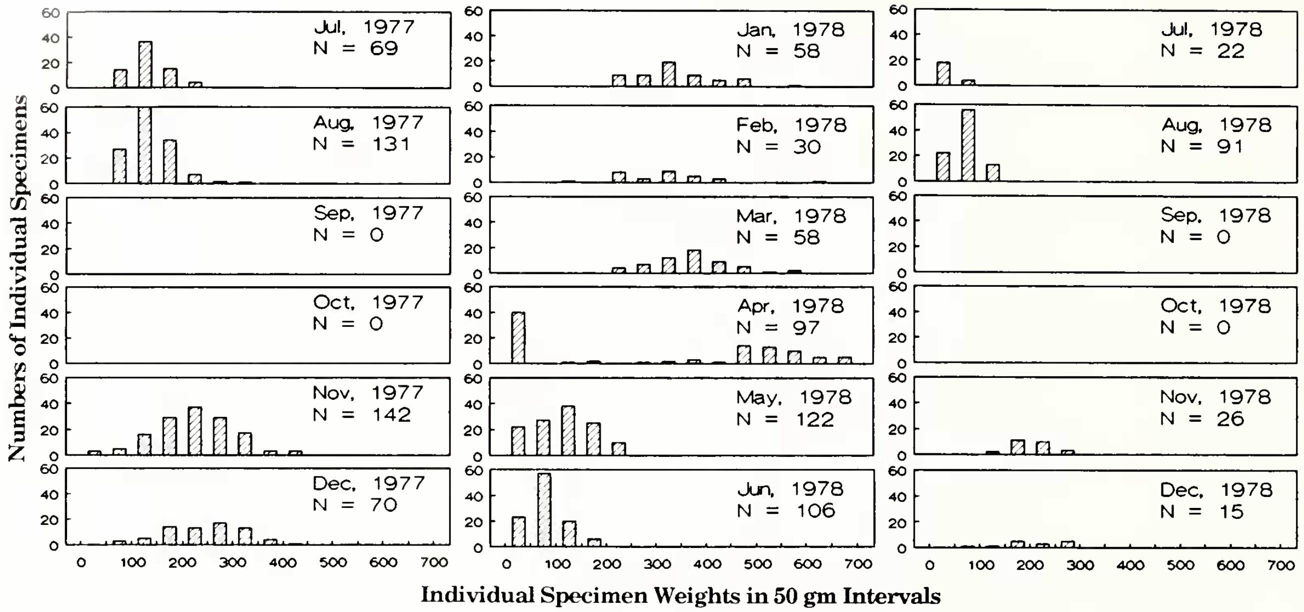

Fig. 1. Numbers of specimens of *Aplysia brasiliana* in each 50 gm weight class of monthly collections at South Padre Island, Texas from July, 1977 through December, 1978.

the population was characterized by specimens in the 250 - 600 gm range. The marked appearance of large numbers of recently recruited juveniles in the April, 1978 collection, as well as moderate numbers of large specimens in the 450 - 700 gm size, clearly revealed the overlap of generations. These very large specimens were absent from the population by the following month. Similar life cycle lengths have been reported by Carefoot (1967) for *A. punctata* Cuvier, by Audesirk (1979) for *A. californica* Cooper, and by Usuki (1970) for *A. kurodai* (Baba) and *A. juliana* Quoy and Gaimard.

RECRUITMENT

The results of this study (Fig. 1) confirm the presence of a major period of recruitment of recently metamorphosed juveniles during the spring of the year. The April, 1978 collection clearly revealed the marked appearance of large numbers of specimens in the 1 - 50 gm weight class. The presence of specimens weighing less than 50 gm in the collections of June, July and August of 1978 initially suggested a continuous 5 month period of recruitment. This does not, however, appear to be the case. Based upon field observations made during 1975, 1976 and 1977, the spring recruitment of 1978 appeared unusually large. Hundreds of swimming specimens were observed in May. Small to medium sized specimens were very abundant on the rocks of the jetty and Coast Guard Station.

A marked decline in numbers of specimens was evident by early summer and, despite an extensive collecting effort, the July collection resulted in only 22 specimens. It appears that the unusually large spring recruitment of 1978 was followed by a mid-summer crash in the population. While the June, July and August collections were characterized by specimens under 50 gm, there were no specimens in the 1 - 10 gm range which had characterized the April collection. The small specimens present in the June, July and August collections of 1978 were therefore considered to be spring recruits which were unable to increase in size due to competition, rather than recent recruits to the population. Collections made during the summer of 1977 (Fig. 1) as well as those of 1976, appear to support this conclusion. These collections were characterized by specimens in the 50 - 300 gm range, as well as the absence of smaller specimens in the 1 - 50 gm range. The summer collections of 1977 are therefore considered typical for this species.

The presence of one 12 gm specimen as well as several others weighing less than 50 gm in the November, 1977 collection (Fig. 1) supports the existence of a minor period of secondary recruitment during the late fall or early winer. The life cycle of *Aplysia brasiliana* at South Padre Island appears to be characterized by the presence of a major period of spring recruitment followed by a very minor period of secondary recruitment during late fall or early winter. Usuki (1970) reported similar findings for *A. kurodai* in the Sea of Japan.

Aplysia brasiliana appears to exhibit a relatively high reproductive potential during much of its life cycle. Upon capture, specimens were observed to engage readily in copulatory and egg laying activities throughout most of the year. Despite this fact, recruitment is clearly not a continual process. Recent life history studies (e.g. Sarver, 1979; Gev *et al.*, 1984) have established the relationship of the seasonal abundance of select algal species with the timing of metamorphosis and subsequent recruitment of juveniles into naturally occurring populations of various aplysiid species (see Carefoot, 1987, for review).

Mature veliger larvae of *Aplysia brasiliana* readily metamorphose in the presence of the red alga *Callithamnion* and, to a lesser degree, *Polysiphonia* (Strenth and Blankenship, 1978b). While *Callithamnion* can be found throughout most of the year (Sorensen, 1979), it reaches its maximum abundance at South Padre Island during March (Penn, 1974). This seasonal abundance of *Callithamnion* just precedes the major period of recruitment of juvenile *A. brasiliana* into the population in April. While many additional environmental factors should be considered, it appears possible that this seasonal peak of *Callithamnion* could be a major contributing factor in the timing of the spring recruitment of juvenile *A. brasiliana* at South Padre Island, Texas.

SEASONAL OCCURRENCE

The disappearance of specimens from otherwise normally occupied habitats during the months of September and October (Fig. 1) appears to be a normal aspect of the life cycle of *Aplysia brasiliana* at South Padre Island. Krakauer (1969) also failed to collect specimens of this species in Florida during this same time period. Studies on other aplysiids have reported similar decreases in numbers of specimens during the fall of the year. Audesirk (1979:413) reported a "nearly total disappearance of animals" for *A. californica* during October, November and December. Gev *et al.* (1984:69) reported that "The *Aplysia* season ends in September" for *A. depilans* Gmelin and *A. fasciata* Poiret along the Mediterranean coast of Israel. Both Audesirk (1979) and Gev *et al.* (1984) related this noticeable drop in numbers of specimens during the fall of the year to the decline of one year class, which in turn is replaced by the succeeding generation.

Results obtained during the course of this study do not support the above premise as it relates to *Aplysia brasiliana* at South Padre Island. While some minor recruitment could occur during November or December following the absence of collectable fall specimens, there is no major shift in modal weight class during the fall such as that observed for the month of April, 1978 (Fig. 1). In addition, average weights of specimens increased from August to November during both years. The average weight during 1977 increased from 139 gm (N = 131) in August to 220 gm (N = 142) in November. During 1978, the average weight increased from 67 gm (N = 91) in August to 206 gm (N = 26) in November. The demonstrated presence of continual weight increase in specimens from August to November, as well as the absence of large numbers of recently recruited juveniles in the early winter collections, serve to support the existence of a standing population of *A. brasiliana* which is characterized by slight to moderate increases in size of individual specimens rather than a population undergoing decline and replacement.

A suitable explanation to account for the location of specimens during September and October does not appear forthcoming from observations made during the course of this study. While discounting reproductive migration in general, Carefoot (1987:204) states that the theory of feeding migrations is often "attractive in that it accounts for seasonal gaps in abundance." Neither the theory of reproductive migration nor that of feeding migration appear to provide a feasible explanation (see below) to account for the disappearance of *Aplysia brasiliana* at South Padre Island during the early fall of the year.

Aplysia brasiliana is a known burrower (Aspey and Blankenship, 1976). Individual specimens could be undergoing continual or prolonged intermittent periods of burrowing in the soft substratum of the south Laguna Madre during September and October. It should be emphasized that this hypothesis is conjectural and not supported by field work. It should be noted, however, that decreased foraging activities by burrowed *A. brasiliana* during September and October could possibly facilitate a rebound in the standing crop of *Callithamnion*, which in turn could account for the secondary period of recruitment during late fall or early winter.

MIGRATION

In his review of migration theory as it relates to life cycles of aplysiids, Carefoot (1987:203) states that "This theory of migration has fallen into disfavour from lack of supporting evidence". While individual specimens were observed to exhibit localized movements in association with foraging behavior, the current study provides no support for the migration theory as it relates to *Aplysia brasiliana* at South Padre Island, Texas. This could be due in part to the somewhat confined nature of the south Laguna Madre as well as the presence in the lagoon of both adult food (*Laurencia* and *Gracilaria*) as well as metamorphosing substrata (*Callithamnion* and *Polysiphonia*) for developing larvae. Juveniles and adults were collected from the exact same habitats during different times of the year.

While specimens of *Aplysia brasiliana* are found occasionally beached on the Gulf of Mexico side of the island, the nature of the offshore substrate does not appear to favor the attachment of suitable benthic marine algae. Consequently, the immediate offshore environment of South Padre Island appears to offer little if any favorable habitat for completion of all or part of the life cycle of *A. brasiliana*. While not surveyed in this study, deeper off-shore reefs are known habitats (Tunnell and Chaney, 1970) for *A. brasiliana* and could provide for variations in life cycles not observed during the course of this study.

CONCLUSIONS

The results of this study are consistent with and support the conclusions of both Krakauer (1969) and Hamilton *et al.* (1982) that the maximum length of the life cycle of *Aplysia brasiliana* is approximately one year. This species exhibits a major period of recruitment during the spring followed by a marked loss of large overwintering specimens from the population. A minor period of secondary recruitment can occur during late fall or early winter. The reason for the absence of collectable specimens during the early fall currently remains obscure.

ACKNOWLEDGMENTS

The presentation of the results of this study during the American Malacological Union meeting at Woods Hole was made possible by a Faculty Development Grant to one of us (N.E.S.) from Angelo State University. Field studies were supported by a National Science Foundation grant (BBS 8711368) to one of us (J.E.B.). Appreciation is extended to the faculty and staff of the Pan American University Marine Laboratory at South Padre Island, Texas. This study would not have been possible without their support. A very special thanks goes to Barbara Strenth for preparation of the figure.

LITERATURE CITED

Aspey, W. P. and J. E. Blankenship. 1976. *Aplysia* behavioral biology:II. Induced burrowing in swimming *A. brasiliana* by burrowed conspecifics. *Behavioral Biology* 17:301-312.

Audesirk, T. E. 1979. A field study of growth and reproduction in *Aplysia californica*. *Biological Bulletin* 157(3):407-421.

Carefoot, T. H. 1967. Studies on a sublittoral population of *Aplysia punctata*. *Journal of the Marine Biological Association of the United Kingdom* 47:335-350.

Carefoot, T. H. 1987. *Aplysia:* Its biology and ecology. *Annual Review of Oceanography and Marine Biology* 25:167-284.

Gev, S., Y. Achituv and A. J. Susswein. 1984. Seasonal determinants of the life cycle in two species of *Aplysia* found in shallow waters along the Mediterranean Coast of Israel. *Journal of Experimental Marine Biology and Ecology* 74:67-83.

Hamilton, P. V., B. J. Russell and H. W. Ambrose. 1982. Some characteristics of a spring incursion of *Aplysia brasiliana* into shallow water. *Malacological Review* 15:15-19.

Krakauer, J. M. 1969. The ecology of *Aplysia willcoxi* Heilprin at Cedar Key, Florida. Master's Thesis, University of Florida, Gainesville, Florida, U.S.A. 87 pp.

Miller, M. C. 1960. A note on the life history of *Aplysia punctata* Cuvier in Manx waters. *Proceedings of the Malacological Society of London* 34:165-167.

Penn, G. J. 1974. Seasonal periodicity of dominant, benthic, marine macroalgae of the north jetty of Brazos Santiago Pass, Texas, as related to environmental factors. Master's Thesis, Pan American University, Edinburg, Texas, U.S.A. 45 pp.

Sarver, D. J. 1979. Recruitment and juvenile survival in the sea hare *Aplysia juliana* (Gastropoda: Opisthobranchia). *Marine Biology* 54:353-361.

Sorensen, L. 1979. *A Guide to The Seaweeds of South Padre Island, Texas*. Gorsuch Scarisbrick Publishers. Dubuque, Iowa, U.S.A. 123 pp.

Strenth, N. E. and J. E. Blankenship. 1978a. On the valid name of the common Texas and Florida species of *Aplysia* (Gastropoda, Opisthobranchia). *Bulletin of Marine Science* 28(2):249-254.

Strenth, N. E. and J. E. Blankenship. 1978b. Laboratory culture, metamorphosis and development of *Aplysia brasiliana* Rang 1828 (Gastropoda: Opisthobranchia). *Veliger* 21(1):99-103.

Tunnell, J. W. and A. H. Chaney. 1970. A checklist of the mollusks of Seven and One-half Fathom Reef, Northwestern Gulf of Mexico. *Contributions in Marine Science* 15:193-203.

Usuki, I. 1970. Studies on the life history of Aplysiidae and their allies in the Sado district of the Japan Sea. *Science Reports of Niigata University, Series D (Biology)* 7:91-105.

Date of manuscript acceptance: 21 December 1990

Sententia

Variation in sense organ design and associated sensory capabilities among closely related molluscs

P. V. Hamilton

Department of Biology, University of West Florida, Pensacola, Florida 32514, U. S. A.

Abstract. Knowledge of ontogenetic and interspecific variation in structural and functional properties of an organ is prerequisite to establishing valid generalizations about the organ's role within a group. However, for many molluscan sense organs, generalizations have become established with minimal knowledge of such variation. Review of data on the gross structure, optical properties and visual responses of 44 gastropods provides a clear example of this problem. Lens structures range from crystalline to gelatinous, with concomitant differences in refractive index, and degree of structural and optical homogeneity. Refractive index measurements from differential interference microscopy indicate that gastropod lenses could be partly or completely corrected for spherical aberration. Photoreceptor separation distances vary from about 3 to 25 μm, and photoreceptor abundances from about five to 100,000 per eye. Estimates of anatomical resolution vary from 0.25 to 14 degrees, and overlap considerably with values for arthropods and vertebrates. Visual responses range from simple taxis to the ability to detect an object's orientation. Reviews of data on the structure and function of opisthobranch rhinophores, and on scallop eye structure, also reveal greater variation than is typically appreciated. More attention must be given to variation in molluscan sense organ structure and function in order for this field to develop more fully.

The field of molluscan biology has been expanding its horizons over the past quarter century from its earlier emphasis on studies of taxonomy, shell morphology and anatomy (Solem, 1974), to greater exploration of life histories, ecological relationships, and behavior. Although few scientists with formal training in animal behavior specialized in studying molluscs 20 years ago, this is no longer true. Most animal behaviorists are still attracted to the more rapidly moving arthropods and vertebrates, but cephalopods have always presented a unique challenge, and the value of certain gastropods as neuroethological models has attracted numerous neurobiologists and some behaviorists, especially those interested in a reductionist approach. A computer search of literature citations dealing with the behavior of molluscs clearly demonstrates the rapid expansion of this subdiscipline (Fig. 1). More than 7,600 citations in Biological Abstracts have included information about molluscan behavior or sensory biology over the past 20 years, and the current rate of increase is about 500 citations per year.

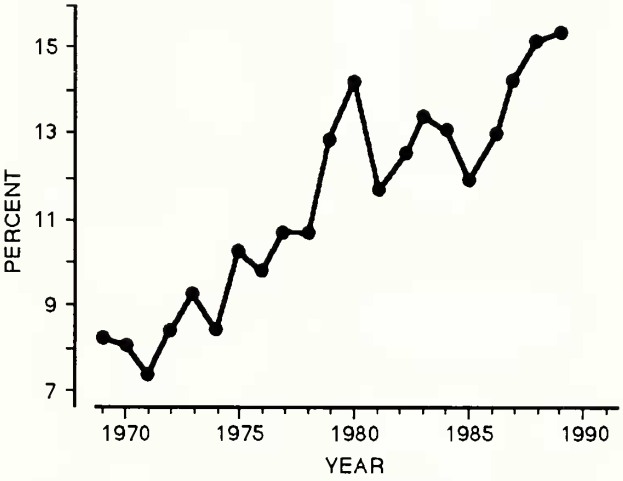

Fig. 1. Number of publications dealing with the behavior or sensory biology of molluscs, as a percentage of all publications dealing with molluscs, over the past two decades. These data are based on a computer search of Biological Abstracts, and should be considered only an approximation of the general trend.

The usual pattern of development in many biological subdisciplines is for initial studies to be conducted on one or two easily obtained species thought to be typical of a group, and for initial generalizations to be developed for the group based on the findings obtained for these 'type' species. Later, as is required for any field to mature, significant efforts must be directed toward consideration of a broad range of species. Information from comparative studies provides opportunities to determine the phylogenetic limits of plasticity in the group, and often leads to revision of generalizations for the group and reconsideration of whether the initially studied species are indeed typical of the group.

Despite the substantial body of information already acquired on the behavior and sensory biology of molluscs, this

American Malacological Bulletin, Vol. 9(1) (1991):89-98

knowledge base is limited in several critical ways. Much of the information contained in the 7,600 citations noted above concerns just a handful of species, and the levels of intrageneric and intrafamilial variation have been hardly examined. In other cases intragroup variation has been examined and found to be considerable, but this does not seem widely appreciated. And finally, some of the 'type' species recognized by early investigators of molluscan behavior and sensory biology do not seem particularly representative in the context of the broader base of knowledge available today. Collectively, these factors would limit the validity of generalizations made about the behavior and sensory biology of any animal group. However, this is especially true for molluscs, as they have undergone an extensive adaptive radiation into a wide range of habitats, and they exhibit substantial plasticity in sense organ structure and behavior (Seed, 1983; Audesirk and Audesirk, 1985).

It seems, therefore, that substantially more attention needs to be given to the variation that exists within molluscan groups in the areas of behavior and sensory biology in order to resolve these problems and allow this field to achieve a more mature level of development. The goal here is to convince the reader of that viewpoint by examining three selected examples involving a range of taxonomic levels. First, eye structure and the behavioral function of vision in the entire Class Gastropoda will be reviewed in some detail. Then, briefer comparisons will be made of the structure and apparent behavioral function of a pair of head tentacles termed 'rhinophores' in the gastropod Subclass Opisthobranchia, and of the structure of the eyes in the bivalve Family Pectinidae (scallops).

GASTROPOD EYES AND VISUALLY MEDIATED BEHAVIOR

The primary example involves vision in gastropod molluscs. It has been traditional for zoologists (e.g. Messenger, 1981) to recognize three structural grades of eyes among the Gastropoda:

a) the open cup or pit eye, in which the intraocular space is unfilled and not isolated from the surrounding medium by a cornea (e.g. *Patella*);

b) the filled cup eye, in which a gelatinous (and hence low refractive index) material fills the intraocular space, but in which a cornea is lacking (e.g. *Haliotis*);

c) the closed cup eye, in which a soft or hard lens is present in the intraocular space, and in which a cornea is present (e.g. *Nerita, Strombus, Littorina*).

Recognition of three grades of structure in gastropod eyes can describe, at best, but three points within what is a wide range of ocular designs. Furthermore, because two of the three traditionally recognized grades are exhibited only by quite primitive gastropods (e.g. *Patella, Haliotis*), this three grade scheme cannot reflect adequately the range of variation present in gastropod eyes.

Land (1981, 1984) and Cronin (1988) have reviewed certain aspects of eye structure and optical properties in invertebrates, or particularly in molluscs, and limited efforts have been made to tabulate data on gastropod eyes (Zunke, 1978; Messenger, 1981; Land, 1984). Although many excellent studies have been made of retinal ultrastructure and neurophysiological responses, these studies have tended to provide incomplete or no information about whole eye structure or, importantly, the eye as a complete optical system. Thus, there are fewer published data than some might suspect on which to base any general conclusions about gastropod vision. A more detailed comparative view of eye structure in this group is provided here (Table 1) by pooling most of the published data, which covers about 32 species, with previously unpublished data on an additional 12 species.

Few authors who have studied gastropod eyes have reported retinal surface areas, or photoreceptor separation distances, abundances, and densities. Thus, most of the data in Table 1 are derived from my computations based on illustrations and data included in the references cited. Average intraocular diameter is the mean of the major axis (distance from pupil to retina along optic axis) and the minor axis (distance between opposite retinal surfaces along a line perpendicular to the major axis and mid-way along it). Retinal areas were computed based on an ellipsoidal model, using the major and minor axes described above, with subtraction of that portion of the ellipsoid's total surface area corresponding to the aperture, where no photoreceptors are located. In most cases, photoreceptor separation distances were obtained directly from an author's measurements or illustrations. However, for *Pterocera (=Lambis) lambis* (Linné) and *Aplysia californica* Cooper, the separation distances used here are 1 μm greater than the indicated photoreceptor diameters; this adjustment corresponds to a minimal thickness of supportive cells separating adjacent receptors. Receptor knowledge base is limited in several critical ways. Much of the information contained in the 7,600 citations noted above concerns just a handful of species, and the levels of intrageneric and intrafamilial variation have hardly been examined. In other cases intragroup variation has been examined sides whose length equals the receptor separation distance). A hexagonal model matches the actual spatial positions of photoreceptors seen in ideal sections taken tangential to the inner retinal surface, for a variety of gastropods (e.g. Hamilton *et al.*, 1983). Receptor densities were computed from retinal area and receptor abundance values. For lens sizes, a single value is given for spherical lenses, and the order of the two values listed for oblong lenses represents the lengths parallel and perpendicular to the optic axis, respectively. Shell length was used as a measure of body length, except in species where the shell is clearly reduced or absent. The length

Table 1. Variation in eye structure among selected gastropod molluscs.

Taxon	Adult Length (mm)	Mean Intraoc. Diam.(μm)	Retinal Area (mm^2)	Receptor Separ. Dist.(μm)	Receptor Abund.	Receptor Density (mm^{-2})	Lens Diam. (μm)	Reference/Source
PROSOBRANCHIA								
Haliotis discus Reeve	150	840	1.703	10.0	19665	11550	n/a	Tonosaki (1967)
Turbo castanea Gmelin	16	514	0.555	2.7	89840	161980	385+	a
Neritina reclivata (Say)	15	248	0.161	5.7	5735	35540	235+	a
Littoraria irrorata (Say)	19	233	0.109	4.4	6565	60470	170	Hamilton *et al.* (1983)
Littorina littorea (Linné)	22	143	0.052	12.0	415	8030	110	Newell (1965)
Tectarius muricatus (Linné)	19	178	0.074	4.5	4210	57030	141	a
Strombus luhuanus Linné	40	1184	3.025	6.0	97020	32080	732	Gillary (1974), Gillary and Gillary (1979)
Pterocera lambis (Linné)	90	1023	2.893	12.0	23200	8020	1023	Prince (1955)
Lioplax pilsbryi Walker	28	220	0.121	5.5	4610	38210	165x150	a
Pomacea paludosa (Say)	50	561	0.775	4.0	55940	72170	440x322	a
Elimia curvicostata (Reeve)	15	103	0.028	4.5	1600	57030	112x104	a
Marginella sp.	9	120	0.031	6.5	835	27330	102	a
Nassarius vibex (Say)	13	197	0.085	6.9	2050	24250	150x165	a
Melongena corona (Gmelin)	76	273	0.166	7.9	3075	18510	215x175	a
OPISTHOBRANCHIA								
Bulla gouldiana Pilsbry	50	327	0.285	12.5	2110	7390	300x225	Jacklet and Colquhoun (1983)
Aplysia brasiliana Rang	178	507	0.613	16.0	2765	4510	388x297	a
A. californica Cooper	216	471	0.572	16.0	2585	4520	450x400	Jacklet and Geronimo (1971), Herman and Strumwasser (1984)
Hermissenda crassicornis (Eschscholtz)	—	—	—	—	5	—	35x50	Stensaas *et al.* (1969)
Tritonia diomedea (Bergh)	—	—	—	—	5	—	150x130	Chase (1974)
Mean for 16 nudibranchs	—	—	—	—	8	—	57	Hughes (1970)
PULMONATA								
Melampus bidentatus Say	13	101	0.018	7.4	375	21210	63x100	a
Lymnaea stagnalis (Linné)	28	140	0.050	10.0	585	11610	110x100	Stoll (1973)
Biomphalaria glabrata (Say)	17	212	0.121	14.8	640	5290	163x131	Schall and Baptista (1990)
Strophocheilus sp.	120	350	0.338	23.8	690	2040	291x240	Oswaldo-Cruz and Bernardes (1982)
Euglandina rosea (Férussac)	63	177	0.083	5.0	3825	46210	175x150	a
Helix aspersa Müller	36	231	0.134	6.3	3890	29120	225x200	Eakin and Brandenburger (1975), Brandenburger (1975)
Succinea putris (Linné)	17	97	0.026	14.7	145	5490	60x70	Zunke (1978)
Limax flavus Linné	88	209	0.132	15.0	680	5160	145x175	Kataoka (1975, 1977)
Agriolimax reticulatus Müller	43	94	0.013	20.0	40	3150	87x67	Newell and Newell (1968)

\+ = Additional lens protrusion through aperture. a = Based on author's previously unpublished observations.

values used were those stated in the references indicated in Table 1 or, when authors failed to indicate the sizes of the animals studied, those given as average adult sizes in appropriate basic references.

Starting at the largest scale, one can consider the relative sizes of the eyes in adults of various species. Figure 2 shows the relationship between the mean diameter of the intraocular space and the body length of adults for 26 species distributed among three subclasses. Two points are clear from this analysis. First, the size of the intraocular space varies fairly widely among gastropods, with *Strombus luhuanus* Linné having an average intraocular diameter more than 12 times greater than *Agriolimax reticulatus* Müller. Second, average intraocular diameter is significantly correlated with body length when all three subclasses are pooled together (r=0.43, P=0.030), and for the subclasses Prosobranchia (n=14, r=0.62, P=0.019) and Pulmonata (n=9, r=0.75, P=0.019) when analyzed separately. Opisthobranchs were not analyzed separately because reasonable measures of intraocular diameter are available for only three species. Gastropod eyes can be positioned within a substantial perioptic sinus, as in littorinids (Newell, 1965; Hamilton *et al.*, 1983), or they can be closely surrounded by connective tissue, as in *Aplysia californica* (Herman and Strumwasser, 1984).

As would be expected, lens size (as measured by area in mid-saggital section) is highly correlated with average intraocular diameter (r=0.87, P<0.0001) and the amount of

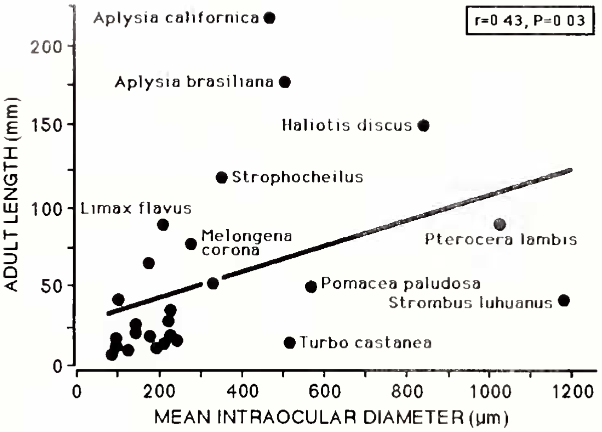

Fig. 2. Relationship between the mean diameter of the intraocular space and the adult body length of 26 gastropods from three subclasses. The species associated with the outlying points are identified. Significant positive correlations also exist for just the prosobranchs (n=14) and just the pulmonates (n=9).

retinal surface area ($r=0.97$, $P<0.0001$). The abalone *Haliotis discus* Reeve lacks a lens, as reportedly do *Trochus* and *Patella* (Hilger, 1885), but the eyes of all other gastropods surveyed here have a lens or lens-like material located in the intraocular space. Optical properties of lenses are influenced partly by their shape. Lens shapes vary from spherical or nearly so, as in the littorinids, strombids and *Lymnaea*, to ellipsoidal, as in various prosobranchs, the aplysiids and most pulmonates (Table 1). Some amount of vitreous material is present between the lens and retina in most species, although Dorsett (1986) suggests this is not the case. Past confusion concerning the presence or absence of vitreous material in the eyes of various species could have been due to the susceptibility of vitreous material to dissolution during histological processing (Charles, 1966; Hamilton *et al.*, 1983).

The lens of *Aplysia californica* was originally illustrated as being spherical, and filling the entire intraocular space (Jacklet *et al.*, 1972). This report led logically to the conclusion that its eye could not form a sharp image (Messenger, 1981; Dorsett, 1986) because of insufficient distance for light rays to be brought to focus on the retina (Land, 1981). However, the illustration in Jacklet *et al.* (1972) is based apparently on an earlier section of the eye in which no aperture was visible, indicating that the section was oblique rather than longitudinal (Jacklet, 1969). A more recent study by Herman and Strumwasser (1984) has shown clearly that the *A. californica* lens and intraocular space are quite ellipsoidal, as is the case in *A. brasiliana* Rang. This finding will hopefully stimulate reconsideration of the visual capabilities of this species.

In addition to these general shape patterns, the lenses of some gastropods exhibit unique shape variants. The opisthobranch *Navanax (=Aglaja) inermis* (Cooper) possesses a distinctly bilobed lens, the function of which is unknown (Eskin and Harcombe, 1977). In the eye of *Turbo castanea* Gmelin, a portion of the lens protrudes through the aperture, and this protrusion of the lens has a shorter radius of curvature than the main body of the lens located within the intraocular space. Because the focal length of a curved refractive surface is directly proportional to its radius of curvature, the protruding portion of lens causes the entire *T. castanea* lens to have a shorter focal length than it would otherwise have, making focus of light on the retina more likely. A wider field of view should also result from this 'fish-eye' lens, but this is probably less significant for *T. castanea*. Similar lens protrusions were described for *Turbo creniferus* Kiem. and *Nerita polita* Linné (Hilger, 1885), and *Neritina reclivata* (Say) also possesses a distinct lens protrusion.

Gastropod lenses vary considerably in hardness. The literature contains numerous pictures of shattered lenses, and it appears that hard lenses are more common than soft lenses in gastropods. However, exact description of lens hardness is difficult, and besides apparent lens hardness (as indicated by degree of shattering when sectioned) seems to be influenced somewhat by the fixative and embedding medium used when processing eyes for histological examination (Hamilton *et al.*, 1983). Hardness is generally correlated with refractive index, a physical property directly relevant to vision. Authors have frequently noted a concentric pattern of stain uptake by gastropod lenses (e.g. Newell, 1965; Jacklet and Colquhoun, 1983; Gibson, 1984), and have inferred from this that such lenses vary concentrically in composition, and presumably refractive index. Gibson (1984) reported that polyhedral subunits, apparently composed of protein, were packed more densely towards the lens center in *Ilyanassa obsoleta* (Say).

Refractive index patterns or gradients within lenses or other structures can be measured exactly from frozen sections using differential interference microscopy. Land has successfully employed this technique with various invertebrates (e.g. Land and Burton, 1979), although data for the lenses of gastropods have not previously been published. In this technique, the distance that an interference fringe is displaced at any given point depends upon the refractive index at that point, as well as light wavelength and specimen thickness, both of which can be controlled.

Preliminary data for the marsh periwinkle, *Littoraria irrorata* (Say), reveal a distinct refractive index gradient within its spherical lens, which is only 170 μm in diameter (Fig. 3). [Reid (1986) moved this species from the genus *Littorina*.] This gradient closely matches the theoretical gradient required for complete correction of spherical abberation (the curve in Fig. 3; based on Fletcher *et al.*, 1954), a major source of potential image degradation. Preliminary data obtained for the ellipsoidal *Aplysia brasiliana* lens also indicate a refractive index gradient, ranging from about 1.40 at the periphery to about 1.51 at the core. The *Turbo castanea* lens varies in

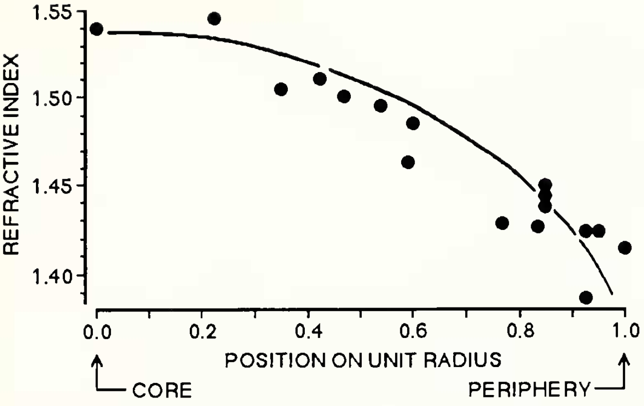

Fig. 3. Refractive index values at different points in the 170 μm diameter spherical lens (f/r=2.71) of the marsh periwinkle, *Littoraria irrorata*, as measured using differential interference microscopy. The curve indicates the refractive index gradient required for perfect correction of spherical aberration in a spherical lens (f/r=2.70) surrounded by a medium whose refractive index is 1.365, which is the average value of cornea and vitreous material.

refractive index from 1.40 at the cornea to 1.45 near the lens center, so even soft lenses can have refractive index gradients. The optical significance of a refractive index gradient is unclear for species with relatively soft lenses, and lenses with non-spherical surfaces. Substantial spherical aberration could exist in such eyes, or spherical aberration could be eliminated if corneal surfaces are parabolic, instead of spherical. In addition to reducing spherical aberration, refractive index gradients also produce a shorter-than-expected focal length, a result which could be as or more important in some species.

Gastropod eyes also vary substantially in retinal and photoreceptor properties (Table 1). Nudibranchs must be considered separately because they clearly exhibit extreme reduction of the eye. For example, Hughes (1970) found that 16 species of nudibranchs had an average of only eight (8) photoreceptors per eye. A survey of the 26 non-nudibranch species on which data are available reveals that retinal areas vary from 0.013 mm^2 *(Agriolimax reticulatus)* to 3.025 mm^2 *(Strombus luhuanus)*, a factor of about 230 times. Most gastropods' photoreceptors contain few or no melanin pigment granules, and so contrast strongly with supportive cells. The photoreceptor counts or estimates reported in the literature have been obtained by various methods. My data on photoreceptor size and spacing have been obtained from sections tangential to the inner retinal surface. As shown in Table 1, adjacent photoreceptors are separated by distances varying from 2.7 μm *(Turbo castanea)* to 23.8 μm *(Strophocheilus* sp.) Photoreceptor abundances per eye vary from 40 *(Agriolimax reticulatus)* to 97,020 *(Strombus luhuanus)*, a factor of about 2,500 times. These photoreceptor abundance values should be viewed only as estimates because at least some gastropods possess two or more receptor types, and these may not be easily distinguished at the light microscope level. Also, photoreceptor densities are known to differ in different regions of the retina in *Littoraria irrorata* (Hamilton *et al.*, 1983) and *Aplysia californica* (Herman and Strumwasser, 1984).

Variation in lens or retinal properties can even be substantial within a family or genus. The greatest variation encountered thus far appears to be among the littorinids. When compared with *Littorina littorea* (Linné), *Littoraria irrorata* has twice the retinal area and a three times shorter receptor separation distance, which results in almost 16 times more receptors per eye (Table 1). *L. irrorata* is active in air, while *L. littorea* appears principally active when submerged in water. That difference in behavioral ecology is probably associated with the substantial difference in eye structure, because the degree to which light is refracted at the cornea depends greatly upon the refractive index of the surrounding medium. *Tectarius muricatus* (Linné), another littorinid, is active in air. Although it has a retinal area intermediate between the other two littorinids, it has a receptor separation distance and total receptor abundance that are much more similar to the littorinid active in air, *L. irrorata.* These similarities in retinal design are related presumably to the higher light levels present in air. Unfortunately, the data available for the eye of *Littorina scutulata* Gould (Mayes and Hermans, 1973) do not allow evaluating the eye as an optical system.

Variation in eye structure in littorinids can be explained by differences in behavioral ecology, but the situation is less clear in other cases of variation between closely related gastropods. Within the Strombidae, *Strombus luhuanus* and *Pterocera (=Lambis) lambis* have similarly sized eyes, but *S. luhuanus* has twice as closely spaced receptors and hence four times more receptors than *P. lambis.* The receptor which Prince (1955) illustrated for *P. lambis* is 6 μm in diameter; however, he indicated that the average receptor diameter is 11 μm, and his estimate of total receptor density ("something approaching" 10,000/mm^2) generally agrees with the estimate in Table 1, which is based on a receptor separation distance of 12 μm. Both strombid species are active in shallow water. *S. luhuanus* is about half the size and travels almost nine times more slowly than *Pterocera (=Lambis) lambis* (Berg, 1974), yet the *S. luhuanus* eye seems capable of resolving greater detail. Berg (1974), who studied ten strombids, noted specifically that *S. luhuanus* seemed "to be able to sense the position" of a predatory cone snail, but he did not speculate on the sensory modality involved. No obvious differences are apparent in eye structure between the two *Aplysia* species listed, beyond the disagreement mentioned earlier about shape of intraocular space and lens. This is somewhat surprising because the species seem to exhibit basic behavioral differences. *A. brasiliana* is an excellent swimmer and is active principally at night. In contrast, *A. californica* apparently does not swim at all, and is diurnally active in the lab and field; whether it may also be nocturnally active under natural conditions remains unknown (Hamilton, 1986; Leonard and Lukowiak, 1986).

Vision in various species can most accurately be compared, not by any of the numbers in Table 1, but rather by knowing the resolving power of the eye. Several factors can influence resolution, but one useful estimate of resolution is the angular separation of adjacent receptors relative to the 'optical center' of the eye (the posterior or proximal nodal point). Resolution measures or estimates have been published for *Littorina littorea* (Newell, 1965), *Littoraria irrorata* (Hamilton *et al.*, 1983), *Strombus luhuanus* (Land, 1984), *Haliotis discus* (Land, 1981), *Biomphalaria glabrata* (Say) (Schall and Baptista, 1989), and *Strophocheilus* sp. (Oswaldo-Cruz and Bernardes, 1982), and reasonable estimates can be computed for a few other gastropods. These resolution values are given in figure 4, along with comparative data for selected arthropods and vertebrates from Kirschfeld (1976). Clearly the resolving powers possessed by gastropod eyes exhibit a wide range, and they overlap considerably with the resolving powers of arthropod and vertebrate eyes.

As Audesirk and Audesirk (1985) suggests, the assumption that vision plays only a minor role in gastropod behavior has had an inhibitory influence on careful studies of visually-mediated behavior in this group. It is commonly believed that the structurally-simple eyes of gastropods only mediate simple phototaxis or skototaxis, orientation toward or away from light or dark areas, respectively. However, critical experiments have rarely been done that could allow discrimination between true phototaxis or skototaxis, and form vision, however crude or simple. Hopefully, the existing collection of anecdotes and weakly founded assertions about gastropod vision will eventually be replaced with carefully obtained data delineating their visual capabilities.

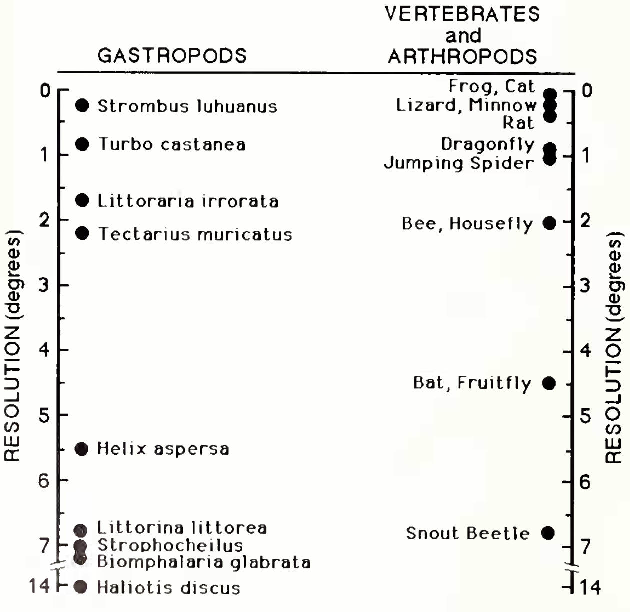

Fig. 4. Measures and estimates of anatomical resolution for nine species of gastropod, with comparative data for selected vertebrates and arthropods.

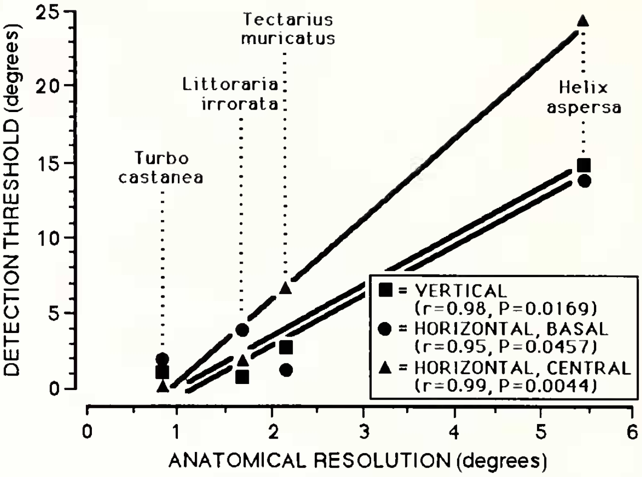

Fig. 5. Measures and estimates of anatomical resolution, and behavioral 'detection' thresholds for oriented responses to three types of targets (vertical stripe, centrally-positioned horizontal stripe, basally-positioned horizontal stripe), for four species of gastropod.

Standardized behavioral measures of visual detection have been obtained for four of the gastropod species discussed here (Hamilton and Winter, 1982, 1984), and these compare well with the previously mentioned anatomical resolution measures and estimates (Fig. 5). Also, it is clear that at least *Littoraria irrorata* can distinguish details of an object's orientation; it preferentially moves toward a vertical black stripe on a white background when presented with horizontal or diagonal stripes having equivalent width and contrast (Hamilton and Winter, 1982). Because the *L. irrorata* eye is not qualitatively different from that of many other gastropods, it could well be that other species can distinguish such visual detail too. Both *Tectarius muricatus* and *Turbo castanea* show some ability to discriminate target orientation (Hamilton and Winter, 1984). An unblocked view of the sky is required for *Aplysia brasiliana* to maintain its swimming direction, which suggests sensitivity to complex visual cues (Hamilton and Russell, 1982a).

In summary, the assortment of optical tricks encountered in the eyes of various vertebrates, including fish eye lenses and lenses with refractive index gradients, are also found among gastropods. This high degree of variation in gastropod eyes should not be viewed as counter-intuitive. The fishes, for example, are less diverse in habitat and general morphology than the gastropods, yet their eyes exhibit an extensive range of adaptations correlated with habitat and behavioral strategy (Lythgoe, 1980; Fernald, 1988). As Land (1981) has noted, there is no clear break within the range of resolving powers exhibited by the eyes of animals. There really is no such thing as an image forming eye as distinct from a non-image forming eye. There are only degrees of need for visual detail among animals, and degrees of image quality provided by eyes. For gastropods and other invertebrates, assumptions about vision, based on vertebrocentric biases, need to be replaced by more hard data and a genuine comparative perspective.

OPISTHOBRANCH RHINOPHORES

This is one of the most obvious cases of unappreciated variation in molluscan sensory biology, and a classic example of why biologists should avoid assigning names to structures that are based on assumed functions. The rhinophores are a pair of tentacles located near the eyes on the dorsal surface of the head, and hence in the same location as the pair of tentacles termed the 'cephalic tentacles' in prosobranch gastropods. The name 'rhinophore' literally means bearer of the nose or nasal sense. This name appears to have been coined by Bergh (1879), who worked principally with the predatory nudibranchs, a group in which the rhinophores do seem involved in distance chemoreception in many species. MacFarland's (1966) treatise on opisthobranchs beautifully illustrates the structural diversity of nudibranch rhinophores. In many species, rhinophoral sheaths are present, as well as numerous lamellae projecting laterally from a central axis. The greatly increased surface area provided by the lamellae is itself suggestive that nudibranch rhinophores have a chemosensory function in this carnivorous group.

However, if one takes a more comparative approach, and looks at other opisthobranch taxa, it is clear that other feeding strategies exist, and that the so-called rhinophores can have a variety of different structural features and sensory functions. In the herbivorous *Aplysia*, for example, each rhinophore has a simple gross structure, with no basal sheath and no lamellae. A pigmented groove on the distal half of the rhinophore receives most of the innervation (Fig. 6; from Ronan, 1990). Despite this much simpler gross structure, aplysiid rhinophores have been implicated in chemoreception, mechanoreception (touch, waves, currents) and even photoreception (Frings and Frings, 1965; Jahan-Parwar, 1972; Audesirk, 1975; Chase, 1979; Jacklet, 1980; Hamilton and Russell, 1982b). It could be that variation exists in rhinophore sensory function, even among *Aplysia* species, since substantial variation exists in morphology, activity rhythms and swimming behavior among aplysiids (Eales, 1960; Hamilton, 1986).

Inadequate appreciation for the variation in rhinophore morphology, feeding strategy and key behavioral traits between nudibranchs and other opisthobranchs has led to the incorrect assumption that the sensory functions associated with the rhinophores of a predatory group (the nudibranchs) automatically apply to other opisthobranch groups. A general under-appreciation for diversity within opisthobranchs could be partly responsible for this problem, but the literal meaning of 'rhinophore' is probably the primary factor. Beginning ethologists are taught the importance of selecting names for the discrete behaviors included in an ethogram based on spatial and temporal features of the movement pattern involved, rather than on the presumed adaptive function of the behavior. For example, the cyclic lateral movement of the siphon which appears when various neogastropods become alerted to a prey's proximity would be named something like 'siphon waving' rather than 'odor searching'. Anatomists are presumably taught some similar rule, and certainly the 'issue' of opisthobranch rhinophores would be less confused today if such a standard had been followed in the 1800's. The confusion that the term rhinophore has caused over the years suggests that we could be better off just referring to these opisthobranch structures as cephalic tentacles, as we do for the seemingly homologous paired tentacles of prosobranch gastropods. Admittedly, the term 'tentacle' suggests a tactile sensory function, but mechanoreception seems a general property of virtually all such structures, regardless of what additional sensory capability they can exhibit.

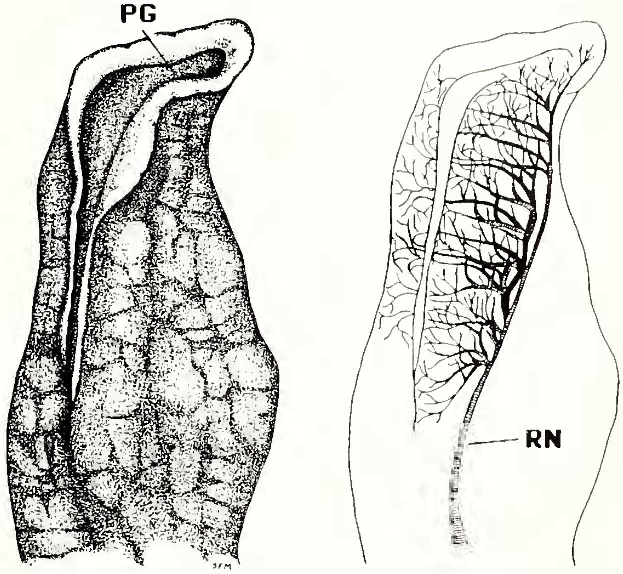

Fig. 6. External view showing the pigmented groove (PG) of the *Aplysia brasiliana* rhinophore (left), and internal view showing the rhinophoral nerve (RN) and its innervation pattern (right), based on analysis of serial sections (from Ronan, 1990, with permission). A fully extended rhinophore is 15-20 mm long in adults.

SCALLOP EYES

Scallops (family Pectinidae) are swimming bivalve molluscs which possess about 60-100 eyes distributed along the mantle edges. The detailed studies of Land (1965) on *Pecten maximus* (Linné) revealed the presence of a unique double-layered retina in each eye, and an equally unique optical system dependent upon a reflector at the rear of the eye, whose surface is described as spherical. Importantly, Land's analyses show little or no space between the rear of the lens and the retina, or between the retina and the reflective argentea. This is in contrast to Dakin's (1928) earlier study of *Pecten maximus*, which reported a space between the lens and retina about 20% of the eye's length. Butcher (1930) found no space between the lens and retina of *Pecten (=Argopecten) gibbus* (Linné) but he found a space occupying about 40% of the eye's length between the retina and argentea of this species. In a preliminary study of the eye of *Argopecten irradians* (Lamarck), Wooters (1989) found a space having a similar size and location to that reported by Butcher, and an argentea which appeared more parabolic than spherical. Finally, considerable variation exists in the sizes of the eyes possessed by individuals in at least some scallop species. This variation could be due to differences in developmental or regenerative growth, but this appears unstudied with the exception of Butcher's (1930) work on *Pecten (=Argopecten) gibbus*. Perhaps the apparent interspecific differences in scallop eye structure are due to different investigators describing eyes at different growth stages in different species.

As with gastropod molluscs, any genuine interspecific differences in scallop eye structure could also be associated with differences in behavior or ecology. *Argopecten irradians* are found in water less than 4 m deep, particularly in beds of the seagrass *Thallasia*, whose blades reach as high as 0.4 m above the bottom. In contrast, *A. gibbus* and *Pecten maximus* are both found in water deeper than 10 m, typically on substrata consisting of sand and shell fragments. Hence, these three scallops differ in the amount of biologically relevant visual detail in their environments, as well as in water depth and associated light characteristics. How these habitat differences or behavioral traits could be associated with differences in eye structure is unknown. Wooters (1969) found that *A. irradians* orient visually toward grassbeds when released in sand patches adjacent to grassbeds, but little is known of the behavioral role of vision in other scallops.

In summary, detailed comparative studies need to be completed to determine exactly what ontogenetic and interspecific variation exists in scallop eye structure and optical properties. Although the eyes of the various scallop species seem generally similar, the available information on eye structure suggests that some interspecific differences could exist in optical properties, especially as regards focal point position and the degree of spherical aberration. Insufficient attention to interspecific variation can easily lead to confusion, and possibly even errors in interpretation. For example, McReynolds and Gorman (1970) studied the electrophysiological properties of the eye of *Pecten (=Argopecten) irradians*, but included a "*Pecten* eye" diagram based on Dakin's (1928) illustration of the eye of *Pecten maximus*. It seems prudent to exercise caution in extrapolating structural, physiological and behavioral results across scallop species until detailed comparative studies are conducted.

CONCLUSION

This review of gastropod vision, opisthobranch rhinophores and scallop eyes demonstrates that there exists considerable variation in sensory system design and capabilities among molluscs, even within families or genera. In the case of gastropod eyes, considerable progress has been made in documenting substantial variation, and the problem seems that the observed variation is underappreciated. In the case of scallop eyes, interspecific variation has barely been documented, yet preliminary results encourage detailed comparative study in the future. The case of opisthobranch rhinophores seems somewhat intermediate; appreciation that the function implied by the name rhinophore might not apply to many opisthobranchs has existed at least since Arey (1918), yet little progress has been made toward obtaining a satisfactory comparative view of the behavioral and ecological correlates of the variations in structure found in this group.

Additional evidence of substantial interspecific variation will likely be obtained eventually for other sense organs, such as the statocyst and osphradium. Statocyst structure has been examined for a modest number of molluscs (Budelmann, 1988), but the possible behavioral-ecological correlates of observed structural diversity have hardly been explored. The literature contains some interesting and apparently paradoxical cases which beg for analysis. For example, the benthic gastropod *Pomacea paludosa* (Say) possesses about 3,000 sensory cells in its statocyst, while a mere 13 cells are present in the statocyst of *Aplysia limacina* de Blainville (=*A. fasciata* Poiret), which is one of the many aplysiids which has been reported to swim as well as crawl on the bottom (Dijkgraaf and Hessels, 1969; Stahlschmidt and Wolff, 1972). Analysis of osphradial variation and its basis across molluscs is complicated by the fact that the name osphradium has sometimes been assigned to structures that are clearly not homologous.

The presence of this substantial interspecific variation emphasizes the necessity of communicating about particular organisms and sense organs more carefully and, where possible, more exactly. The limits and bases of variation would be more easily understood if more authors would include specific information about the sizes of the actual animals they studied, as well as the relationship between sense organ dimensions and body size in the species. Zunke's (1978) study of the eye of *Succinea* includes detailed coverage of ontogenetic variation and, unfortunately, it is unusual in doing so. Failure to consider intraspecific or ontogenetic variation can lead to generalizations just as premature and flawed as those based on too few or atypical species. Autrum (1979) pointed out that it is clearly a bad habit to speak just of "the fly'' in scientific writings. Likewise, it can be a bad habit to speak just of ''the littorinid'', ''the rhinophore'' or ''the scallop'', depending on what species, system or capability is being discussed.

Finally, as more comparative data become available, it should become possible to discard some of the less informative terms in general use for sensory structures. For example, there have existed in the invertebrate literature several terms for structurally simple eyes. 'Pigment cup eyes' and 'pinhole eyes', which both lack lenses, have widely accepted definitions based on structural features (see Land, 1981). However, 'eyespot' and 'ocellus' both mean ''a very small simple eye formed in invertebrates.'' As has so often been the case in invertebrate zoology, the emphasis seems to have been on establishing the existence of a difference as compared to the vertebrate eye, rather than on recognizing the various invertebrate eyes as being distinct structures worthy of independent study and understanding.

As a relatively new field, many gaps exist in our knowledge of molluscan behavior and sensory biology. A clearer understanding would be provided about sensory capabilities within groups if more species were examined, and this would also allow more thorough investigation of the basis of existing variation across species. However, more effort should also be devoted to completing the data sets for species about which some information is already available. In many cases, only data on structure of sense organs or sensory tissues are available from which to draw inferences about function; obviously the availability of data from electrophysiological and behavioral studies would be desirable in such instances. For example, scallop eye structure and electrophysiological responses have received some study, but there is little understanding of how well they can see or what adaptive value vision has in nature. A reasonably complete range of data are available for only a few cases, mostly involving species that serve as model systems for neurobiologists. Thus, future researchers in molluscan sensory biology should be encouraged to flesh out existing stories, as well as to expand our understanding of variability within the group by examining as-yet-unstudied species.

ACKNOWLEDGMENTS

I thank W. Jones for assistance in computing partial surface areas of ellipsoids, S. Miller for drawing figure 6, and C. N. D'Asaro and J. S. Wooters for assistance and critical comments. Portions of this research were supported by NSF grants BNS79-16358 and BNS83-08186, and by an award from UWF's research committee. This is contribution 91-4 of the Institute for Coastal and Estuarine Research.

LITERATURE CITED

Arey, L. B. 1918. The multiple sensory activities of the so-called rhinophores of nudibranchs. *American Journal of Physiology* 46:526-532.

Audesirk, T. E. 1975. Chemoreception in *Aplysia californica*. 1. Behavioral localization of distance chemoreceptors used in food finding. *Behavioral Biology* 15:45-55.

Audesirk, T. and G. Audesirk. 1985. Behavior of gastropod molluscs. *In: The Mollusca*, Volume 8, A. O. D. Willows, ed., pp. 1-94. Academic Press, New York.

Autrum, H. 1979. Preface. *In: Handbook of Sensory Biology*, Volume VII/6A, H. Autrum, ed. pp. v-vii. Springer-Verlag, Berlin.

Berg, J. C. 1974. A comparative ethological study of strombid gastropods. *Behaviour* 51:274-322.

Bergh, R. 1879. On the nudibranchiate gasteropod Mollusca of the north Pacific Ocean, with special reference to those of Alaska. *Proceedings of the Academy of Natural Sciences* 1879:71-132, 1880:40-127.

Brandenburger, J. L. 1975. Two new kinds of retinal cells in the eye of a snail, *Helix aspersa*. *Journal of Ultrastructure Research* 50:216-230.

Budelmann, B-U. 1988. Morphological diversity of equilibrium receptor systems in aquatic invertebrates. *In: Sensory Biology of Aquatic Animals*, J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga, eds. pp. 757-782. Springer-Verlag, New York.

Butcher, E. O. 1930. The formation, regeneration, and transplantation of eyes in *Pecten (gibbus borealis)*. *Biological Bulletin* 59:154-164.

Charles, G. H. 1966. Sense organs (less cephalopods). *In: Physiology of Mollusca*, Volume II, K. M. Wilbur and C. M. Yonge, eds. pp. 455-521. Academic Press, New York.

Chase, R. 1974. The electrophysiology of photoreceptors in the nudibranch mollusc, *Tritonia diomedia*. *Journal of Experimental Biology* 60:707-719.

Chase, R. 1979. Photic sensitivity of the rhinophore in *Aplysia*. *Canadian Journal of Zoology* 57:698-701.

Cronin, T. W. 1988. Vision in marine invertebrates. *In: Sensory Biology of Aquatic Animals*, J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga, eds. pp. 403-418. Springer-Verlag, New York.

Dakin, W. J. 1928. The eyes of *Pecten*, *Spondylus*, *Amussium* and allied lamellibranchs, with a short discussion on their evolution. *Proceedings of the Royal Society of London, B* 103:355-365.

Dijkgraaf, S. and H. G. A. Hessels. 1969. Über bau und funktion der statocyste bei der schnecke *Aplysia limacina*. *Zeitschrift für vergleichende Physiologie* 62:38-60.

Dorsett, D. A. 1986. Brains to cells: the neuroanatomy of selected gastropod species. *In: The Mollusca*, Volume 9, A. O. D. Willows, ed. pp. 101-187. Academic Press, New York.

Eakin, R. M. and J. L. Brandenburger. 1975. Understanding a snail's eye at a snail's pace. *American Zoologist* 15:851-863.

Eales, N. B. 1960. Revision of the world species of *Aplysia* (Gastropoda: Opisthobranchia). *Bulletin of the British Museum of Natural History, Zoology* 5:268-404.

Eskin, A. and E. Harcombe. 1977. Eye of *Navanax*: optic activity, circadian rhythm and morphology. *Comparative Biochemistry and Physiology* 57A:443-449.

Fernald, R. D. 1988. Aquatic adaptations in fish eyes. *In: Sensory Biology of Aquatic Animals*, J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga, eds. pp. 435-466. Springer-Verlag, New York.

Fletcher, A., T. Murphy and A. Young. 1954. Solutions to two optical problems. *Proceedings of the Royal Society of London, A* 223:216-225.

Frings, H. and C. Frings. 1965. Chemosensory bases of food-finding and feeding in *Aplysia juliana* (Mollusca: Opisthobranchia). *Biological Bulletin* 128:211-217.

Gibson, B. L. 1984. Cellular and ultrastructural features of the adult and the embryonic eye in the marine gastropod, *Ilyanassa obsoleta*. *Journal of Morphology* 181:205-220.

Gillary, H. L. 1974. Light-evoked electrical potentials from the eye and optic nerve of *Strombus:* Response waveform and spectral sensitivity. *Journal of Experimental Biology* 60:383-396.

Gillary, H. L. and E. W. Gillary. 1979. Ultrastructural features of the retina and optic nerve of *Strombus luhuanus*, a marine gastropod. *Journal of Morphology* 159:89-116.

Hamilton, P. V. and M. A. Winter. 1982. Behavioural responses to visual stimuli by the snail, *Littorina irrorata. Animal Behavior* 30:752-760.

Hamilton, P. V. and B. J. Russell. 1982a. Celestial orientation by surface-swimming *Aplysia brasiliana* Rang (Mollusca: Gastropoda). *Journal of Experimental Marine Biology and Ecology* 56:145-152.

Hamilton, P. V. and B. J. Russell. 1982b. Field experiments on the sense organs and directional cues involved in offshore-oriented swimming by *Aplysia brasiliana* Rang (Mollusca: Gastropoda). *Journal of Experimental Marine Biology and Ecology* 56:123-143.

Hamilton, P. V., S. C. Ardizzoni and J. S. Penn. 1983. Eye structure and optics in the intertidal snail, *Littorina irrorata. Journal of Comparative Physiology* 152:435-445.

Hamilton, P. V. and M. A. Winter. 1984. Behavioural responses to visual stimuli by the snails *Tectarius muricatus, Turbo castanea*, and *Helix aspersa. Animal Behavior* 32:51-57.

Hamilton, P. V. 1986. Swimming tracks of *Aplysia brasiliana*, with discussion of the roles of swimming in sea hares. *Veliger* 28:310-313.

Herman, K. G. and F. Strumwasser. 1984. Regional specializations in the eye of *Aplysia*, a neuronal circadian oscillator. *Journal of Comparative Neurology* 230:593-613.

Hilger, C. 1885. Beitrage zur Kenntnis des Gastropodenauges. *Morphologisches Jahrbuch* 10:351-371.

Hughes, H. P. I. 1970. A light and electron microscope study of some opisthobranch eyes. *Zeitschrift für Zellforschung und mikroskopische Anatomie* 106:79-98.

Jacklet, J. W. 1969. Electrophysiological organization of the eye of *Aplysia. Journal of General Physiolgy* 53:21-42.

Jacklet, J. W. and J. Geronimo. 1971. Circadian rhythm: population of interacting neurons. *Science* 174:299-302.

Jacklet, J. W., R. Alvarez and B. Bernstein. 1972. Ultrastructure of the eye of *Aplysia. Journal of Ultrastructure Research* 38:246-261.

Jacklet, J. W. 1980. Light sensitivity of the rhinophores and eyes of *Aplysia. Journal of Comparative Physiology* 136:257-262.

Jacklet, J. W. and W. Colquhoun. 1983. Ultrastructure of photoreceptors and circadian pacemaker neurons in the eye of a gastropod, *Bulla. Journal of Neurocytology* 12:673-696.

Jahan-Parwar, B. 1972. Behavioral and electrophysiological studies on chemoreception in *Aplysia. American Zoologist* 12:525-537.

Kataoka, S. 1975. Fine structure of the retina of a slug, *Limax flavus* L. *Vision Research* 15:681-686.

Kataoka, S. 1977. Ultrastructure of the cornea and accessory retina in a slug, *Limax flavus* L. *Journal of Ultrastructure Research* 60:296-305.

Kirschfeld, K. 1976. The resolution of lens and compound eyes. *In: Neural Principles in Vision*, F. Zettler and R. Weiler, eds. pp. 354-372. Springer-Verlag, Berlin.

Land, M. F. 1965. Image formation by a concave reflector in the eye of the scallop, *Pecten maximus. Journal of Physiology* 179:138-153.

Land, M. F. and F. A. Burton. 1979. The refractive index gradient in the crystalline cones of the eyes of a euphausiid crustacean. *Journal of Experimental Biology* 82:395-398.

Land, M. F. 1981. Optics and vision in invertebrates. *In: Handbook of Sensory Physiology,* Volume VII/6B, H. Autrum, ed. pp. 471-594. Springer-Verlag, Berlin.

Land, M. F. 1984. Molluscs. *In: Photoreception and Vision in Invertebrates*, M. A. Ali, ed. pp. 699-725, Plenum Press, New York.

Leonard, J. L. and K. Lukowiak. 1986. The behavior of *Aplysia californica* Cooper (Gastropoda: Opisthobranchia): I. Ethogram. *Behaviour* 98:320-360.

Lythgoe, J. N. 1980. *The Ecology of Vision.* Clarendon Press, Oxford. 244 pp.

MacFarland, F. M. 1966. Studies of Opisthobranchiate Mollusks of the Pacific Coast of North America. *Memoirs of the California Academy of Science,* Vol. VI, 546 pp..

Mayes, M. and C. O. Hermans. 1973. Fine structure of the eye of the prosobranch mollusk *Littorina scutulata. Veliger* 16:166-168.

McReynolds, J. S. and A. L. F. Gorman. 1970. Photoreceptor potentials of the opposite polarity in the eye of the scallop, *Pecten irradians. Journal of General Physiology* 56:376-391.

Messenger, J. B. 1981. Comparative physiology of vision in molluscs. *In: Handbook of Sensory Physiology,* Volume VII/6C, H. Autrum, ed. pp. 93-200. Springer-Verlag, Berlin.

Newell, G. E. 1965. The eye of *Littorina lilttorea. Proceedings of the Zoological Society of London* 144:75-86.

Newell, P. F. and G. E. Newell. 1968. The eye of the slug *Agriolimax reticulatus* (Mull.). *Symposium of the Zoological Society of London* 23:97-111.

Oswaldo-Cruz, E. and R. F. Bernardes. 1982. Morphological and functional observations on the eye of *Strophocheilus* (Mollusca, Gastropoda, Stylommatophora). *Brazilian Journal of Medical and Biological Research* 15:161-174.

Prince, J. H. 1955. The molluscan eyestalk: using as an example, *Pterocera lambis. Texas Reports on Biology and Medicine* 13:323-339.

Reid, D. G. 1986. The littorinid molluscs of mangrove forests in the Indo-Pacific region: the genus *Littoraria. British Museum (Natural History), London.* 227 pp.

Ronan, M. P. 1990. A review of opisthobranch rhinophores with a study of *Aplysia* rhinophore structure. Master's Thesis, University of West Florida, Pensacola, Florida. 113 pp.

Schall, V. T. and D. F. Baptista. 1989. Structural and functional analysis of the eye of *Biomphalaria glabrata* (Mollusca, Gastropoda, Basommatophora). *Brazilian Journal of Medical and Biological Research* 22:497-508.

Seed, R. 1983. Structural organization, adaptive radiation, and classification of molluscs. *In: The Mollusca*, Volume 1, P. W. Hochachka, ed. pp. 1-54. Academic Press, New York.

Solem, G. A. 1974. *The Shell Makers.* John Wiley, New York. 289 pp.

Stahlschmidt, V. and H. G. Wolff. 1972. The fine structure of the statocyst of the prosobranch mollusc *Pomacea paludosa. Zeitschrift für Zellforschung und mikroskopische Anatomie* 133:529-537.

Stensaas, L. J., S. S. Stensaas and O. Trujillo-Cenóz. 1969. Some morphological aspects of the visual system of *Hermissenda crassicornis* (Mollusca: Nudibranchia). *Journal of Ultrastructure Research* 27:510-532.

Stoll, C. J. 1973. Observations on the ultrastructure of the eye of the basommatophoran snail, *Lymnaea stagnalis* (L.). *Proceedings of Koninklijke Nederlandse Akademie van Wetenschappen*, Ser. C, 76(4):1-11.

Tonosaki, A. 1967. Fine structure of the retina in *Haliotis discus. Zeitschrift für Zellforschung und mikroskopische Anatomie* 79:469-480.

Wooters, J. S. 1989. The ecology of vision in the bay scallop, *Argopecten irradians* (Pectinidae: Bivalvia). Master's Thesis, University of West Florida, Pensacola, Florida. 57 pp.

Zunke, U. 1978. Bau und Entwicklung des auges von *Succinea putris* (Linné, 1758) (Mollusca, Stylommatophora). *Zoologischer Anzeiger* 3:220-244.

Date of manuscript acceptance: 16 April 1991

Research Note

Sperm storage and evidence for multiple insemination in a natural population of the freshwater snail, *Physa*

Amy R. Wethington and Robert T. Dillon, Jr.[1]

Department of Biology, College of Charleston, Charleston, South Carolina 29424, U. S. A.

Abstract. Although a number of estimates are available regarding the capacity of freshwater pulmonates to store sperm after laboratory mating, few such data are available for wild-collected snails, where the recency of mating is necessarily unknown. We collected 35 *Physa heterostropha pomilia* (Conrad) from a local population and held each in isolation for 60 days, rearing all egg masses. We then used protein electrophoresis to determine the LAP genotype of each parent and a sample of its offspring. Five of the parental snails (total 60 day fecundities from 300 - 600 progeny) were found to be homozygous at the LAP locus yet producing approximately 50% heterozygous offspring. In 4 of these 5 cases, no significant difference was detected in offspring genotype frequencies over 60 days, suggesting both that mating has generally been recent and that reservoirs of stored sperm are, as a rule, large. In the fifth case, the frequency of heterozygous offspring increased significantly, suggesting multiple insemination. Multiple insemination and sperm storage have obvious adaptive significance to colonizing species such as freshwater pulmonates.

The capability to store sperm, especially sperm contributed by multiple partners, has the potential to lessen the severity of genetic drift by increasing the effective population size represented by a small number of survivors or colonizers. And the capacity of an individual pulmonate snail to store sperm can be prodigious. In *Bulinus*, laboratory mating has been reported to provide enough sperm to fertilize from 1000 - 2000 eggs up to 4400 eggs, depending particularly upon the reproductive condition of the snail acting as female (Rudolph, 1983; Rudolph and Bailey, 1985). In *Biomphalaria*, longevity of stored sperm has been variously estimated as 25 - 68 days (Paraense, 1955), 42 days (Richards, 1973), and more than 100 days (Monteiro *et al.*, 1984). Vianey-Liaud *et al.* (1987) reported a mean of about 50 days with a range from 3 days to 127. Cain (1956) reported some exogenously fertilized egg production by *Lymnaea stagnalis* Say up to 116 days after isolation. All these studies have involved lab crosses with pigment variants (usually albinism) as a genetic marker. Rollinson and Wright (1984) used isozyme markers to demonstrate sperm storage up to 70 days after laboratory mating of Mauritian *Bulinus*.

The effect of a delay in oviposition by the mother (as by a severe winter or desiccation on a birds foot, for example) has been investigated by Rudolph and Bailey (1985). Apparently *Bulinus* can store viable exogenous sperm through a minimum of seven weeks of starvation, eight weeks of low temperature (10 - 15°C) or four weeks of desiccation.

Rudolph and Bailey (1985) also reported some fairly strong evidence that *Bulinus* can store sperm from more than a single male simultaneously. Although short-lived copulatory plugs have been described in several pulmonate species, behavioral observations nevertheless suggest that multiple insemination could be common in laboratory situations (Rudolph, 1979a, b; van Duivenboden and ter Maat, 1988). Duncan (1959) reported that he had not observed reciprocal copulation in either *Physa fontinalis* (L.) or *P. acuta* (Drap.). But we have observed both reciprocal copulation and multiple mating behavior in our laboratory populations of *Physa*, without genetic markers for confirmation.

Using isozyme markers, Mulvey and Vrijenhoek (1981) found strong evidence of multiple paternity in clutches of eggs laid by isolated wild-caught *Biomphalaria*. It could not be determined, however, if the sperm used to fertilize these eggs came from two different exogenous sources, or from a combination of one outcross and selfing. It does in fact seem that fertilization can proceed with endogenous and exogenous sperm simultaneously, at least in some situations (Paraense, 1955; Monteiro *et al.*, 1984; Rollinson, 1986). But multiple insemination has been conclusively documented by Rollinson *et al.* (1989), using stocks of *Bulinus cernicus* (Morelet) homozygous for three different alleles at the Gpi locus.

As important as they are, data from lab crosses such as these do not directly address the likelihood of genetic drift after a population crash or founder event. A second set of

---

[1] Address correspondence to RTD

variables is involved: how recently will an arbitrarily chosen pulmonate snail have mated? On the average, how healthy and fecund will it be? Rollinson (1986) has performed experiments bearing on this question using several species of African *Bulinus*. He isolated individual wild-caught snails and collected and reared their eggs up to 60 days. He then examined isozyme phenotype of both parent and offspring at four polymorphic loci. The large majority of these parents did lay demonstrably outcrossed eggs, apparently using stored sperm. Stored sperm was used for at least 41-44 days. Intriguingly, Rollinson found one *Bulinus scalaris* (Dunker) clearly producing offspring from two different fathers.

The great majority of all observations to date have involved lab matings of the tropical planorbids *Bulinus* and *Biomphalaria*. So to test the generality of the sperm storage phenomenon in natural populations of temperate pulmonates, we performed an analysis similar to Rollinson's on a local population of *Physa*.

METHODS

Physa heterostropha pomilia (Conrad) is a widespread and variable species, found throughout eastern North America (Wurtz, 1949; Burch and Tottenham, 1980). Recently Te (1978, 1980) has suggested that this taxon, along with the majority of North American species, be separated into the resurrected genus *Physella*. But we agree with Taylor (1988) that the distinction between *Physella* and *Physa* (s.s.) is more properly at the rank of subgenus.

We collected *Physa heterostropha pomilia* from a pond at Charles Towne Landing, a state park within the city limits of Charleston, South Carolina. The snails did not appear to be common on this nor on any subsequent trip, and we felt it possible that individual encounters and matings could be infrequent in this population. Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia.

For an initial survey of polymorphism, we homogenized 62 snails in a 7% sucrose solution, buffered at pH 7.4 with 0.05 M tris (hydroxymethyl) aminomethane and H_3PO_4, to which xylene cyanole had been added as marker. Samples were centrifuged and horizontal starch gel electrophoreses was performed on the supernatant using standard techniques (Dillon and Davis, 1980; Dillon, 1982, 1985). The 14% starch gels were made of 3 parts Sigma starch: 1 part Electrostarch and AP6 buffer, diluted 19:1. AP6 buffer is 0.04 M citric acid (monohydrate) adjusted to pH 6 with N−(-3-aminopropyl) morpholine (Clayton and Tretiak, 1972). Gels were run for approximately 4.5 hr at 40 volts under refrigeration. They were then sliced and stained for leucine aminopeptidase (LAP) using the recipe of Shaw and Prasad (1969).

Two isozymes were found to be segregating in a fashion consistent with Hardy Weinberg expectation in the Charles Towne Landing population, at gene frequencies of 0.58 and 0.42. We have designated the more common isozyme "LAP 100", and the isozyme migrating 3 mm faster in our gel conditions "LAP 103".

We returned to Charles Towne Landing in May, 1989, and collected 35 adult *Physa*, placing each in a separate 10 oz plastic cup of pond water with a plastic petri dish cover. We fed them commercial Tetra-Min "Conditioning" food for plant-eating fish (Jennings *et al.*, 1970), and changed the water with fresh, aerated pond water periodically. Each parent was checked daily and transferred to a new cup when an egg mass was produced, in a fashion similar to that of Rollinson (1986). Water was changed once a week for the adults (along with newly laid egg masses) and once every other week for the juvenile snails when they had grown to a size at which this could be done safely.

The experiment was terminated in July (after 60 days), by which time 29 of the parents had reproduced, some prolifically and others much less so. We then determined the genotypes of each parent and a sample of early laid offspring at the LAP locus. Of the 15 LAP homozygotes identified, four were producing all homozygous progeny and the remainder were producing high frequencies of heterozygotes, obviously using exogenous sperm. We did not find any case where a homozygous parent was producing entirely heterozygous offspring, as could be expected from a single outcross to the opposite homozygote. But we selected for further study the five largest sibships from the 11 including heterozygotes. We then compared the frequency of heterozygotes in 20 to 30 offspring from the earliest egg masses in these sibships to a similar sample from the last laid sibships.

RESULTS

Data on the fecundity of these five snails (A through E) during the 60 day study period are presented in figure 1. The figure shows the cumulative number of juveniles surviving to countable size. It can be seen that all parents continued to lay eggs throughout the entire period, although the rate slowed, especially after day 45. Total viable egg masses ranged from 15 to 19, and the total countable offspring ranged from about 300 to 600.

Table 1 shows the frequency of heterozygotes in 20 to 30 offspring of the first laid sibships and the frequency of heterozygotes in similar sized samples from the last laid sibships. Genotype frequencies were not significantly different from 1:1 (chi square, Yates corrected) in any of the five groups of first laid offspring. This is consistent with Mendelian expectation if each mother had mated with a single heterozygous father.

Table 1 also shows that in four of the five cases, there was no significant difference between the genotype frequencies in the first laid sibships and the last laid sibships.

Table 1. LAP genotypes among the offspring of five parent *Physa*, first laid sibships compared to last laid sibships.

Parent	LAP Genotype	Day Number of Oviposition	Number of Homozygotes	Number of 100/103 Heterozygotes	chi-square
A	103/103	4,9	20	15	1.44
		58,60	16	5	
B	100/100	7,11	16	17	6.63**
		51,52,53,56	2	18	
C	100/100	2,8	17	13	0.36
		56,57,58	11	7	
D	103/103	2	19	15	1.04
		44,51,56	9	14	
E	103/103	7	13	17	0.13
		51,52,56	12	11	

**P < 0.01

The one significant value of chi square (contingency test, Yates corrected) is shown in the offspring of parent B, where there was an unexpected excess of heterozygotes among the offspring laid in the final days of the experiment.

DISCUSSION

We do not know whether the total fecundities reflected in figure 1 overestimate or underestimate production by a single *Physa* founding a new population in the wild. On the one hand, we attempted to provide food in excess and protected the juveniles from predation. But competition among juveniles could have been an important factor in our plastic cups, especially on occasions when as many as three egg masses were laid overnight.

Although it can be seen that all parents continued to lay eggs for the entire 60 day period, there was a reduction in the number of viable embryos per egg mass through time. For example, the last three egg masses laid by snail E, on days 57, 58, and 60, contained no viable embryos at all. Qualitatively these results are similar to those obtained by Duncan (1959) with English *Physa fontinalis*, although *P. heterostropha pomilia* fecundities seem to be higher. As previous work suggests a gradual shift from exogenous to endogenous sperm (Paraense, 1955; Cain, 1956), we initially interpreted this observation as evidence of depleted sperm stores. But Table 1 shows that after 60 days and as many as 600 offspring, none of these individuals seems to have exhuasted its reservoir of stored sperm. In retrospect, our decision to terminate the experiment was premature.

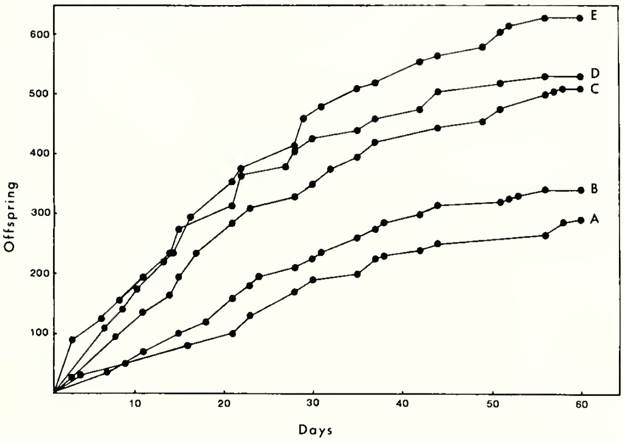

Fig. 1. Cumulative 60-day fecundity (offspring surviving to countable size) of the five *Physa* examined for sperm storage.

The excess of heterozygotes observed among the progeny of parent B is not easily explained as the result of a single pair mating. If the offspring were fathered by a single heterozygous individual, one would expect a 1:1 ratio of homozygous to heterozygous progeny. This is indeed the ratio observed in all early sibships, laid on days 2 through 11 (Table 1). But the sample of snail B progeny from days 51, 52, 53, and 56 contained 18 heterozygotes and only 2 homozygotes, very significantly different from 1:1. Even combining all 53 progeny examined from parent B, one still obtains a significant excess of heterozygotes (goodness-of-fit chi square = 4.79). Nor can these results be explained by a single cross to a homozygous father, as no homozygous progeny would have been expected at all. To argue that self fertilization played any role, one would need to postulate that parent B was at least partly self fertilizing to start but increasingly shifted to exogenous sperm as the days in isolation passed, quite counter to all previous observations on other pulmonates.

By far the most likely explanation for the results from parent B is multiple insemination by both a heterozygote and a homozygote for the opposite allele. The first eggs seem to have been fertilized by sperm from the former, and the last eggs by the latter, in a fashion similar to that inferred for *Biomphalaria* (Mulvey and Vrigjenhoek, 1981) and well documented for *Bulinus* (Rudolph and Bailey, 1985; Rollinson, 1986; Rollinson *et al.*, 1989). Clearly more work is called for, possibly using multiple loci as markers for different parents. But if multiple insemination is in fact widespread, individual pulmonate snails surviving colonization events, hard winters or severe storms could potentially represent a great deal of genetic variation indeed.

Given the apparently large capacity *Physa heterostropha pomilia* displays for sperm storage, it would be interesting to see how frequently an average snail mates, and in what capacity. The great majority of freshwater pulmonate species show either simultaneous development of both reproductive tracts, or are slightly protandric (Russell-Hunter and McMahon, 1976; Rudolph, 1983). The male organs develop before the female organs in *P. fontinalis*, although environmental conditions in the wild do not in general favor mating until both organ systems are mature (Duncan, 1959). It is not clear whether fully mature pulmonates of any sort prefer to mate as a certain sex, whether a snail's sexual role can change with size or sperm stores, or how often snails switch roles in single encounters. Multiple insemination introduces questions of sperm competition and sperm "sharing" (Monteiro *et al.*, 1984; Vianey-Liaud *et al.*, 1987).

ACKNOWLEDGMENTS

We thank Charles Towne Landing State Park and Mike Dorn for access to the *Physa* population and Dr. Margaret Mulvey for her advice on pulmonate culture.

LITERATURE CITED

Burch, J. B. and J. L. Tottenham. 1980. North American freshwater snails: species list, ranges, and illustrations. *Walkerana* 3:1-215.

Cain, G. L. 1956. Studies on cross-fertilization and self-fertilization in *Lymnaea stagnalis appressa* Say. *Biological Bulletin* 111:45-52.

Clayton, J. W. and D. N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* 29:1169-1172.

Dillon, R. T., Jr. 1982. The correlates of divergence in isolated populations of the freshwater snail, *Goniobasis proxima*. Doctoral Dissertation, University of Pennsylvania, Philadelphia. 183 pp.

Dillon, R. T., Jr. 1985. Correspondence between the buffer systems suitable for electrophoretic resolution of bivalve and gastropod isozymes. *Comparative Biochemistry and Physiology* 82B:643-645.

Dillon, R. T., Jr. and G. M. Davis. 1980. The *Goniobasis* of Southern Virginia and Northwestern North Carolina: genetic and shell morphometric relationships. *Malacologia* 20:83-98.

Duncan, C. J. 1959. Life cycle and ecology of the freshwater snail, *Physa fontinalis. Journal of Animal Ecology* 28:97-117.

Jennings, A. L., K. N. DeKock and J. A. von Eeden. 1970. An evaluation of five different kinds of experimental freshwater snail food. *Wetensk Bydraes Potchefstroomse Universitat* B (29):1-14.

Monteiro, W., J. M. G. Almeida, Jr. and B. S. Dias. 1984. Sperm sharing in *Biomphalaria* snails: a new behavioral strategy in simultaneous hermaphroditism. *Nature* 308:727-729.

Mulvey, M. and R. C. Vrijenhoek. 1981. Multiple paternity in the hermaphroditic snail, *Biomphalaria obstructa. Journal of Heredity* 72:308-312.

Paraense, W. L. 1955. Self and cross-fertilization in *Australorbis glabratus. Memorias do Institato Oswaldo Cruz* 53:285-291.

Richards, C. S. 1973. Genetics of *Biomphalaria glabrata* (Gastropoda: Pulmonata). *Malacological Review* 6:199-202.

Rollinson, D. 1986. Reproductive strategies of some species of *Bulinus. In: Proceedings of the Eighth International Malacological Congress,* L. Painter, ed. pp. 221-226. Hungarian Natural History Museum, Budapest, Hungary.

Rollinson, D., R. A. Kane and J. R. L. Lines. 1989. An analysis of fertilization in *Bulinus cernicus* (Gastropoda: Planorbidae). *Journal of Zoology (London)* 217:295-310.

Rollinson, D. and C. A. Wright. 1984. Population studies on *Bulinus cernicus* from Mauritius. Malacologia 25:447-464.

Rudolph, P. H. 1979a. An analysis of copulation in *Bulinus (Physopsis) globosus* (Gastropoda: Planorbidae). *Malacologia* 19:147-155.

Rudolph, P. H. 1979b. The strategy of copulation of *Stagnicola elodes* (Say) (Basommatophora: Lymnaeidae). *Malacologia* 18:381-389.

Rudolph, P. H. 1983. Copulatory activity and sperm production in *Bulinus (Physopsis) globosus* (Gastropoda: Planorbidae). *Journal of Molluscan Studies* 49:125-132.

Rudolph, P. H. and J. B. Bailey. 1985. Copulation as females and use of allosperm in the freshwater snail genus *Bulinus. Journal of Molluscan Studies* 51:267-275.

Russell-Hunter, W. D. and R. F. McMahon. 1976. Evidence for functional protandry in a freshwater basommatophoran limpet, *Laevapex fuscus. Transactions of the American Microscopical Society* 95:174-182.

Shaw, C. R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. *Biochemical Genetics* 4:297-320.

Taylor, D. W. 1988. New species of *Physa* (Gastropoda: Hygrophila) from the western United States. *Malacological Review* 21:43-79.

Te, G. A. 1978. The systematics of the family Physidae (Basommatophora: Pulmonata). Doctoral Dissertation, University of Michigan, Ann Arbor. 325 pp.

Te, G. A. 1980. New classification system for the family Physidae (Pulmonata: Basommatophora). *Archiv für Molluskenkunde* 110:179-184.

Van Duivenboden, Y. A. and A. ter Maat. 1988. Mating behavior of *Lymnaea stagnalis. Malacologia* 28:53-64.

Vianey-Liaud, M., J. Dupouy, F. Lancastre and H. Nassi. 1987. Genetical exchanges between one *Biomphalaria glabrata* (Gastropoda: Planorbidae) and a varying number of partners. *Memorias do Instituto Oswaldo Cruz* 82(4):457-460.

Wurtz, C. B. 1949. *Physa heterostropha* (Say). *Nautilus* 63:2-7.

Date of manuscript acceptance: 10 September 1990